



# COMPARATIVE ASPECTS OF THE PITUITARY GLAND

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AND  
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AS A TOKEN OF RESPECT AND ADMIRATION



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## Foreword

Researches in medical sciences have touched almost every age of mankind. They have provided for the medical men a wide spectrum of useful knowledge. Surgical science has embarked upon new areas, opening up altogether a new horizon. In course of this triumphant march has accumulated new information for solving new problems.

The author has traversed a new domain in his "Comparative Aspects of the Pituitary Gland" and has indicated potential areas of research. Every chapter is replete with examples of his Scholastic approach to the understanding of his numerous research findings. They are results of the author's long experience in this specialised field.

By comparing his work with that of others he has been able to establish findings related to the family of other vertebrates. In this context he has dealt not only with cytological aspects but also discussed the rather fragmentary and incomplete knowledge about certain groups of animals. He has tried to fill up the gaps in the works of earlier authors on the control mechanism of the gland with his own findings on different vertebrates and show new avenues of research.

A brilliant scholar, researcher and teacher, the author has won many distinctions and occupies a position of honour in the field of medical education. I am confident that specialists and advanced research workers will find this book useful. To an author of his eminence nothing will be more rewarding than this.

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1 June 1978.

( S. K. Mukherjee )  
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## P R E F A C E

In my earlier treatise, "Neuroendocrinological studies in stress : experimental surgical observations in vertebrates and invertebrates" published in 1976 by the University of Calcutta I have presented my observations mainly on comparative aspect of hypothalamo-pituitary-adrenal axis mechanism. While doing so the idea of compiling my findings on the comparative aspect of the pituitary gland enriched with the comprehensive review of the relevant observations of the pioneer workers in the field crystallised in my mind. Thus I took up this venture. The volume intends to present collectively the comparative features of the pituitary gland in different vertebrates based on histological, histochemical and ultrastructural studies as undertaken by eminent scientists in the field and by the author. To make the presentation more interesting and cogent I have deliberately included a precise discussion on some of the circumventricular organs because of intimate closeness of the same with the pituitary gland.

I take this privilege to keep on record my sincere gratitude to the late Professors B. Houssay, Dr. B. Romeis, and G. W. Harris, to Professors Hans Selye, K. Lissak, R. Diepen, B. T. Donovan, W. Bargmann, Allen Costoff, G. E. Pickford, M. G. Farquhar, R. L. Holmes, J. N. Ball, A. Tixier-Vidal, C. B. Jorgensen, K. G. Wingstrand, I. Chester Jones, Howard Bern, H. D. Purves, H. Kobayashi, K. Tsuneki, P. G. W. J. van Oordt, E. J. W. Barrington, M. Oliverau, and I. P. Callard from whose work I have made liberal references in comprehending the review of literature. In fact, the thought-provoking works of these esteemed authors have stimulated and encouraged me to undertake this comparative study of the pituitary gland.

Karplus and Kreidl (1909, 1910, 1912) first described that the hypothalamus had some bodily functions. On stimulation of the walls of the third ventricle there was acceleration of the heart-beat, changes in the movement and secretion of the gut, dilatation of the pupils and other autonomic effects. Cerebral hemispheres were removed from many of their experimental animals for destroying the projection fibres. On stimulation of the wall of the third ventricle in such animals the same reactions were found. Local anaesthesia applied to the part to be stimulated, abolished the reactions. They also traced the paths which were essential for the mediation of the response. Cardiac responses depended upon the ventral roots of the upper five or six thoracic segments. Stellate ganglionectomy blocked the response.



Houssay and Molinelli (1925) stated that epinephrin secretion by the adrenal medulla is controlled by the hypothalamus.

Hess (1925, 1932, 1938, 1948, 1954) stressed upon the importance of the hypothalamus and he developed a method for stimulating different parts of the diencephalon in conscious cats by indwelling electrodes. After the stimulation experiments were over, the particular area of the brain was lesioned and the ~~cats were~~ ~~lesioned~~ ~~ed~~.

Cannon's (1932) work on emergency reactions and homeostasis laid importance on the sympathetic nervous system.

In the second half of the nineteenth century Claude Bernard said that one of the most important features of all living beings is their ability to maintain the constancy of their internal *milieu* inspite of changes in the environment.

Selye (1936) attached importance to the pituitary adrenocortical system. The totality of the damage and the body's adaptive reaction has been called by him as Stress Syndrome or General Adaptation Syndrome.

Lissák and Endröczy (1960) stress the importance of the central nervous system in the adaptation activity.

Roussy and Mosinger (1946) in their *Traite de Neuro-Endocrinologie* said that they were forced to demonstrate the existence of a reflex hypophysial neuro-regulation as a result of concentrating their study on the sumtotal afferent nervous paths to the excitosecretory centres of the hypophysis.

With all humility I admit that references of all the workers in this field could not be covered for which I beg sincere apology ; the want of space stood in the way to elaborate many valuable works even.

The historical aspect of the study on the structure and function of the pituitary gland has been excellently reviewed by Professor R. L. Holmes and Professor J. N. Ball in their book "The Pituitary Gland" in 1974. Therefore this part does not need elaboration here.

Earlier in 1958 Professor L. Belloni in his introductory note on *Rete mirabile* summarised the historical observations of the earlier authors and the function of the brain and pituitary prevailing at that time. He said that the anatomicophysiological system of Galen (2nd Century A.D.) a formation is situated in an important place at the base of the cranium around the hypophysial infundibulum called *Rete mirabile* because of an opinion which was accepted in the middle ages. The vital spirit was brought by the blood stream from the left heart through the carotids to this level where the *miraculous* transfer from the vital spirit to the animal spirit took place. In the cerebral cells or cavities there was localization of the higher functions of the soul. Professor Belloni started from this doctrine and reviewed the various phases (sympathetic phase, humoral phase etc.) and ultimately came to the present



idea which means that the diencephalon is the regulating centre of the vegetative life and the emotional life through complex processes of integration with the cerebral cortex and the conscious life. He referred to ancient doctrines on cerebral secretion.

Professor K. M. Knigge (1975) referred to the work of Rene Descartes on *Treatise on Man* about 350 years ago. Descartes thought that different types of *spirits* from the body had an entrance into the brain through the pineal gland. The spirits were distributed through the *Cor* to the anterior and posterior parts of the brain. Different types of mechanisms in the pineal gland regulated the distribution of these spirits to the brain. The gland produced its own spirits which passed into CSF. Descartes in his time did not specify the neural lobe, median eminence, organum vasculosum laminae terminalis, subfornical organ, subcommissural organ and the area postrema as in his time there was no electron microscope. Knigge further said, "These seven specialized circumventricular structures of the mammalian brain represent windows with individualized structural characteristics permitting intimate contact between blood and cerebrospinal fluid, neurons and specialized ependyma-glia. These *seven windows of the brain* like the seven lucky deities of Japan, may each have a specific patron of bodybrain function which they serve".

Recently it has been proved that for the neural control of the anterior pituitary there is neuronal synthesis of peptidic releasing hormones in the hypothalamus and these are stored in the outer part of the median eminence. The releasing hormones are released into the pituitary portal vessels and reach the anterior pituitary for controlling its secretion of different hormones. Now it is thought that the releasing hormones pass into CSF and they pass through the ependymal cells of the median eminence to the pituitary portal vessels.

I feel thrilled to draw the special attention of the learned readers to the description of a concept as envisaged in the practice of *Tantras* and *Yogas* of ancient India which essentially enunciates the modern idea of the brain, ventral hypothalamus, circumventricular organs and cerebrospinal fluid controlling the pituitary gland. This, I strongly feel, needs a little elaboration.

Ancient *Tantras* in India speak that the *spirit* known as *Śakti* is to cross the five centres in the spinal cord which are known as centres or *Cakras* and ultimately come into a particular part or *Sahasrāra* of the brain. Thus the *Cakras* are six in number. Within the *Sahasrāra* there is a nodal area which is the hypothalamus, median eminence and the third ventricle which are the abode of *Parama Śhiva*. Pituitary is the abode of *Śakti* or the spirit. The theory of the *Tāntriks* speaks that *Kundalini* ascends and the human body is maintained by the *nectar* which flows down after the union of *Śiva* and *Śakti* in the *Sahasrāra*. This nectar is formed by their union.



सदाशिवेन देवेशि चणमात्रं रमेत् प्रिये ।

अमृतं जायते देवि तत्क्षणात् परमेश्वरि ।

तदुद्भवामृतं देवि लाचारसमन्वितां ।

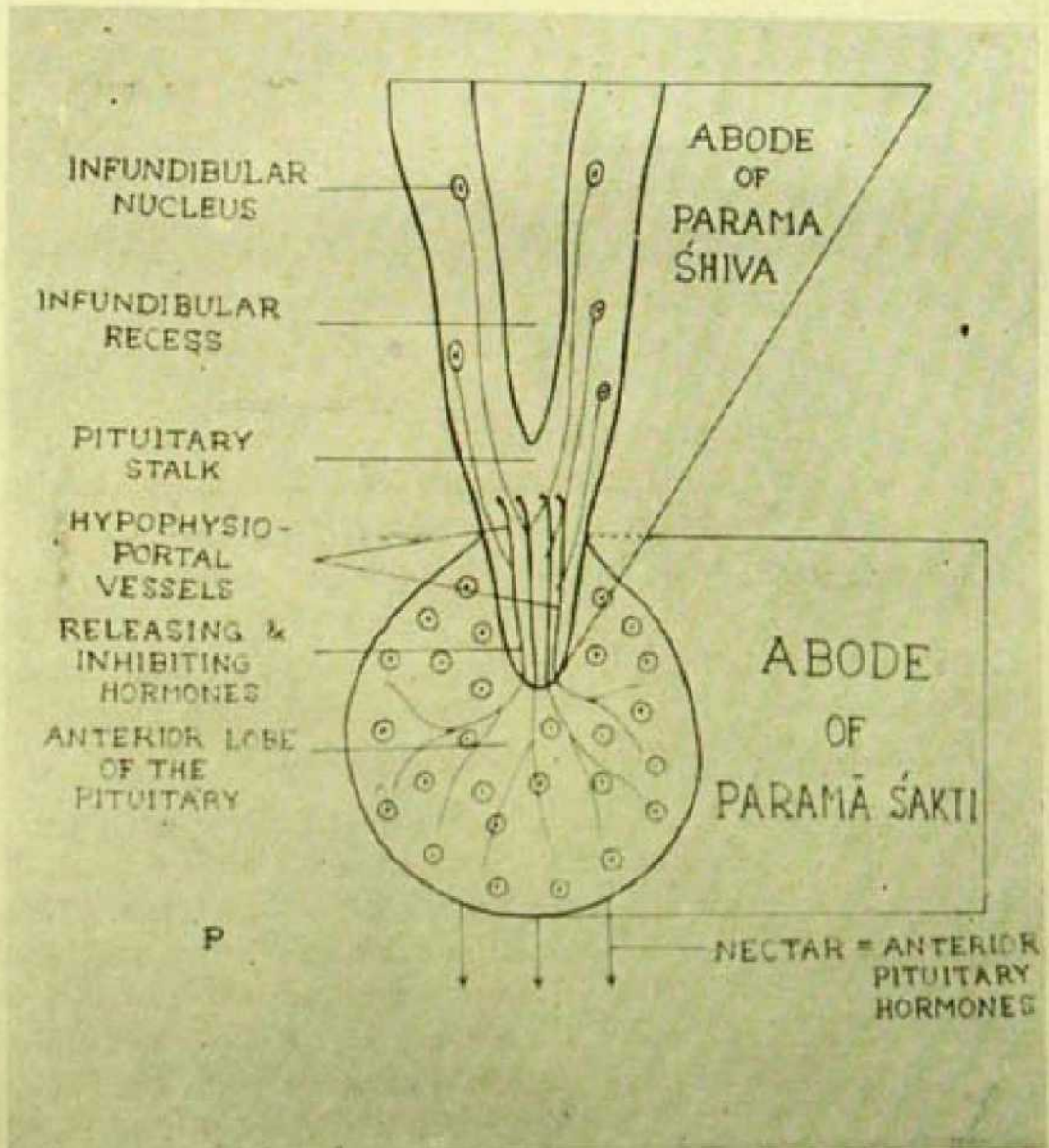
तेनामृतेन देवेशि तर्पयेत् परदेवतां ।

—भूतशुद्धि तन्त्रवचन

This means :

Momentary union between *Śhiva* and *Śakti* results in elaboration of the nectar by the latter. The resultant nectar has the colour of that of the extract of lic. The nectar is responsible for the well-being of the body and the mind.

Compared to modern sciences this area and facts can be depicted in the following diagram :





Late Dr. Shashibhusan Das Gupta in his "Obscure religious cults" has said that the final end of the *Nāth Siddhās* is immortality in a perfect body and in a divine body.

Secretion of nectar from the moon i.e. *Sahasrāra* is associated with the rousing of the *Kundalini Śakti* and it is held that rousing of *Śakti* in the *Sahasrāra* is instrumental to the trickling down of the nectar and sometimes *Śakti* herself is depicted as the drinker of the nectar. Drinking of wine and eating of meat which are indispensable to a *Tāntrick Sādhaka* are explained by the *Nāth Yogins* as drinking of nectar from the moon and turning the tongue backwards in the hollow above.

Control of mind through the control of the wind by *Prāṇāyāma* is very important in the *Nātha* literature. For the final arrest of the mind there are psychological processes from *Āsana* to *Samādhi*. In the *Nāth* cult these processes are associated with the process of retaining the *Mahā-rasa* (nectar) and the *Yogic* regulation of the secretion for the transubstantiation of the body and in this way eternal life is attained.

A curved duct (*Baṅka nāla*) connects the moon (the hypothalamo-hypophysial region) below the *Sahasrāra* (Brain) with the hollow of the palatal region through which the *Soma* or *Nectar* or *Amyta* is poured. This path is reminiscent of Rathke's pouch of the developing pituitary having a communication with the roof of the pharynx and also of Rathke's pouch. In the developing adenohypophysis there is only a residual lumen and the former is connected with the epithelium of the pharynx by an epithelial stalk. The curved duct is known as the *Saṅkhinī* in the *Yoga* physiology. The upper opening of *Saṅkhinī* is known as *Daśama-dvāra* (tenth door) of the body. The *Yogic Sādhana* of the *Nāth Siddhas* veers round the conservation and the *Yogic* regulation of the nectar or the *Mahā rasa*.

The orohypophysial duct persists in the *Polypterus ornatipinnis*. In some juvenile clupeoids (also the herring) and in the young of some other primitive teleosts an orohypophysial duct is present. It is also present in the young of palaeoniscoids. In *Elops* and *Hilsa ilisha* this duct is present in the adult. In the coelacanth (*Latimeria chalumnae*) the Rathke's pouch is unusually long during development and isolated islets of glandular tissue are formed in it. There is a large buccal pars distalis. An epithelial stalk being originated from the oral part of Rathke's pouch persists in many species of birds. This cellular connection stretches between the pars distalis and the oral ectoderm.



The nervous and vascular mechanisms are involved for the mediation of the stress responses from man to the fish. In man and higher vertebrates the reticular formation, hypothalamus, septal nucleus, amygdaloid nucleus, hippocampus, pyriform cortex, cingulate cortex, orbito-insular-temporal cortex, prefrontal cortex are all involved in the mediation of the stress response either in facilitatory or inhibitory ways.

In bird, reptiles, amphibians and fish hypothalamic and extrahypothalamic areas are also involved in the mediation of the stress response.

### **Hypophysiotrophic area**

This area corresponds to the place which is the abode of *Parama Śiva*.

This is the mediobasal hypothalamus (MBH) and CRF activity has been noted in it and in the median eminence. Hypothalamic CRF circadian peak occurs three hours before the peak of corticosterone in the blood.

ACTH secretion can be maintained by anterior pituitary homografts if they are vascularized by the median eminence vessels. Such grafts also respond to stress in the fish, amphibians, reptiles, birds and mammals.

The work presented in this volume has been undertaken during the tenure of Maharaja of Darbhanga Research Scholarship which was offered to the author in 1976-77 by the University of Calcutta.

I express my deep sense of gratitude to Dr. Satyendra Nath Sen, Ex-Vice-Chancellor and Dr. P. K. Bose, Ex-Pro-Vice-Chancellor, University of Calcutta, who inspired me in starting this work. My sincere gratitude is extended to Dr. Sushil Kumar Mukherjee, Ex-Vice-Chancellor, University of Calcutta for his affectionate blessings and kind permission to get this book published by our University. At the same time I express my indebtedness and gratitude to Sri Arun Ray, Pro-Vice-Chancellor for Business Affairs and Finance and Dr. R. K. Poddar, the present Vice-Chancellor of the Calcutta University.

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The royalty accrued from the sale of this book is hereby donated to the University of Calcutta.

The author wishes to place on record his deep sense of appreciation and gratitude to all the pioneer workers who have devoted themselves wholeheartedly to the study of Hypothalamo-Pituitary-Adrenal-Axis which inspired the author more than anything else to take up the present work.

Despite my sincere attempts to make the present volume foolproof a few errors and omissions may have crept in while completing this volume for which responsibility is entirely mine. Such error, if there be any, is very much regretted.

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### **Explanation of abbreviations**

AC=Acid phosphatase

AChE=Acetylcholinesterase (specific)

AD=Aldolase

AK=Alkaline phosphatase

AMPase=Adenosinemonophosphatase (5'-nucleotidase)

AP=Amylophosphorylase

ATPase=Adenosinetriphosphatase

BIUL=Bismuth iodide + uranyl acetate and lead

E-PTA=Ethanollic phosphotungstic acid

GERL=Golgi-endoplasmic reticulum-lysosomal complexes

G-6-PD=Glucose-6-phosphate dehydrogenase

LDH=Lactic dehydrogenase

LERL=Lipid-endoplasmic reticulum-lysosome complexes

MAO=Monoamine oxidase

NAD=Nicotinamide-adenine dinucleotide

SDH=Succinic dehydrogenase

TPPase=Thiamine pyrophosphatase

UDPG=Uridine diphosphoglucose glycogen transferase

असतो मा सद्गमय  
तमसो मा ज्योतिर्गमय  
मृत्योर्माऽमृतं गमयेति ॥

From untruth lead me to Truth ;  
From darkness lead me to Light ;  
From mortality lead me to Immortality.

—*From Brihadāranyaka Upanishad*  
Ch.—I.



Micron ( micrometer ) :  $\mu$  (  $\mu\text{m}$  ) = 0.001 mm,  $10^{-6}\text{m}$

Millimicron ( nanometer ) :  $\text{m}\mu$  ( nm ) = 0.001  $\mu\text{m}$ ,  $10^{-9}\text{m}$

Ångström :  $\text{\AA}$  = 0.1 nm,  $10^{-10}\text{m}$

### **Circumventricular or ependymal organs (Hofer, 1958, 1965)**

These are specialized neuroendocrine structures which seem to be associated with all of the recesses of the midline ventricles. They are highly vascularized. Sex hormone concentrating cells have been demonstrated within these structures or in their close vicinity (Stumpf, 1975). These organs include median eminence, neural lobe, pineal body, subcommissural organ, subfornical organ, organum vasculosum of the lamina terminalis (OVLT), area postrema, organum (recessus) mammillare, and the organum colliculare caudalis.



## CHAPTER 1

### RECENT TRENDS IN THE RESEARCH OF CIRCUMVENTRICULAR ORGANS

Naik(1976) concluded that the LH-RH positive neurons were more in the nucleus of the diagonal band of Broca, the dorsal and the medial septal nuclei, the preoptic, medial prechiasmatic areas, in the ventromedial and arcuate nuclei and in the ventrolateral-premamillary body. In other areas of the hypothalamus surrounding the suprachiasmatic, supraoptic and anterior hypothalamic areas, and the paraventricular nuclei, these nuclei were few and scattered at random. A few neurons could also be traced in the median eminence and in the infundibular stem.

Majority of the LH-RH nerve fibres were found in the external layer of the median eminence and they were found to end around the capillaries of the primary portal plexus of the median eminence. A few fibres terminated near the third ventricle. Thus it is possible that secretion of LH-RH is released mostly in the median eminence portal system and some in the ventricular fluid. Transport of LH-RH from ventricular fluid to the median eminence capillaries occurs through the ependyma. The immunoelectron microscopic findings suggest that the LH-RH is synthesized in the neurons and packaged or condensed in the granules (dense core vesicles) in the Golgi cisternae.

Jonas *et al.*(1976) attempted to measure the concentration of endogenous LH-RH in the peripheral plasma of the sheep in various physiological states. The concentrations were 120-400 pg/ml in five animals. The majority of this material was found to be methanol-insoluble, heat-stable and of low or absent biological activity, unlike synthetic LH-RH. "Levels of immunoreactive LH-RH in methanolic plasma extracts are at the limits of assay sensitivity ( $12 \pm 6$  pg/ml), while assays in plasma concentrates suggest that endogenous levels are 1 pg/ml in most samples".

Kordon(1976) reviewed recent data on cellular aspects of the neuroendocrine hypothalamus and presented new data on hormone-neurotransmitter interaction in gonadotrophic regulation. Nerve terminals containing LH-RH could be detected in the vascular organ of the lamina terminalis, in a broad area of the median eminence and in the dorsal tip of the pituitary stalk. Regarding the origin of LH-RH containing fibres there are two views :—

- (1) the cell bodies could be traced in the arcuate nucleus,
- (2) the cell bodies occupy a wide area of the anterior hypothalamus.



LH-RH distribution in nerve terminals has been shown in subcellular fractionation experiments. Most bioassayable and radioimmunoassayable LH-RH is found in the synaptosomal fraction. Detectable amounts of the peptide could not be recovered from the supernatant fractions containing most soluble material from the perikarya.

LH-RH activity is not found beyond the arcuate-median eminence area and the cell bodies are situated in the medio-basal hypothalamus. There is no terminal degeneration of the neurovascular junctions of the median eminence after anterior hypothalamic deafferentation. This proves that fibres taking origin from the anterior hypothalamic areas do not end at that level. Kordon and his associates (1976) made a Halasz-type cut in the retrochiasmatic region of the hypothalamus. At 15 days after deafferentation, a significant amount of label could be detected in the basomedial hypothalamus. The concentration was mostly in the rostral part of the median eminence and in the dorsal tip of the stalk. The organum vasculosum of the lamina terminalis is anterior to the level of section. LH-RH fibres innervating this organ originate outside medio-basal hypothalamus. Immunoreactivity has not been noted in the perikarya of the rat. This may be due to the fact that the hormone is synthesized as a prohormone in the cells and transported along axons and only to be detected in the special zone of the median eminence where enzymatic activity imparts the biological and immunological properties (Kordon, 1976). An alternative hypothesis suggests that the tanycytes of the basomedial hypothalamus can transfer the peptide from the third ventricle to the portal system.

Kordon (1976) concludes, "our present feeling is that future investigation will focus attention of the cellular and subcellular mechanisms of these regulations. This will probably permit to settle some of the problems concerning the level of action of neurotransmitters, the importance of their respective effects as synthesis, axonal transport and release of neurohormones. Detailed information on the fine actions of steroid or synthetic hormones acting at the hypothalamic level should also be expected from this approach, thus permitting a better screening of compounds which may be useful as fertility or antifertility agents and a better evaluation of their possible side effects.

Pfaff and Keiner (1973) observed in rats, two hours after intraperitoneal injection of oestradiol- $H^3$  that there are oestradiol-concentrating cells in a system of limbic and hypothalamic structures. These cells are present in the medial preoptic area, medial anterior hypothalamus, ventromedial nucleus, arcuate nucleus and ventral premammillary nucleus. Amongst the limbic structures there are medial and cortical nuclei of the amygdala, lateral septum, bed nucleus of the stria terminalis, diagonal band of Broca, olfactory tubercle, ventral hippocampus, and prepiriform and entorhinal cortex. Such cells were also found in the lateral and ventrolateral portions of the mesencephalic central gray. Weak labelled cells are irregularly found in the spinal cord. They conclude that, "the evidence suggests a limbic-hypothalamic system of oestrogen-concentrat-





ing neurons which participate in the control of mating behaviour and of gonadotrophin release from the pituitary".

Pfaff (1976) said that a similarity exists broadly between the neuroanatomic distribution of oestradiol and testosterone but there may be some differences in detail within each individual limbic or hypothalamic structures. The preoptic area and the hypothalamus do not bind steroids indiscriminately.  $^3\text{H}$ -corticosterone is bound strongly by neurons in the hippocampus and is not bound strongly by above mentioned neurons.

The female rat has more oestradiol-binding neurons than the female hamster and their location in the rat is rather further forward in the medial preoptic area than in the medial anterior hypothalamus. Rats are much more sensitive to oestradiol than hamsters. Oestrogen binding has been noted in the medial preoptic area, medial hypothalamus and lateral septum of the female cat. In the Rhesus monkey the locations of oestrogen-binding neurons are in limbic forebrain structures, the preoptic area and the midline hypothalamus. In the male chaffinch (songbird) the song depends upon testosterone. Labelled cells in the lateral septum, the medial preoptic area, and in midline hypothalamic nuclei have been noted in castrated male chaffins with testosterone treatment. In the mesencephalon labelled cells were noted in specific cell grouping called nucleus intercollicularis after injections of  $^3\text{H}$ -testosterone. This nucleus is responsible for vocalization. "Testosterone neurons in the preoptic area mediate androgenic effects on mating behaviour and labelled neurons in the hypothalamus effects in the release of pituitary hormones. In the castrated male Zebra finch labelled cells have been noted in the lateral septum, medial preoptic area, medial anterior hypothalamus and in tuberal hypothalamic nuclei after intramuscular injection of  $^3\text{H}$ -testosterone. Androgen binding was also noted in the mesencephalic nucleus intercollicularis. In the lower brain stem specific androgen binding by the motoneurons which control the syrinx (a specialization of the trachea which is responsible for song production) was also noted."

In the castrated male *Xenopus*  $^3\text{H}$ -testosterone labelled cells were found in the medial preoptic area and in a midline hypothalamic nucleus, the ventral nucleus of the tuber cinereum. Similar binding in the cells are noted in the female *Xenopus*.

Ovariectomized female *Xenopus* showed  $^3\text{H}$ -estradiol accumulation in the cells of the septum, the amygdala and in a specific portion of the striatum. High density of labelled cells was noted in the medial preoptic area and in the ventral nucleus of the tuber cinereum. Small number of labelled cells was found in the ventral thalamus and in the torus semicircularis of the midbrain. The pattern of distribution of the estradiol binding cells in the brain of male *Xenopus* was same as that noted in females.



$^3\text{H}$ -testosterone labelled cells were noted in the anterior pituitary and in the midline hypothalamic nuclei surrounding the pituitary stalk in castrated male sunfish after intraperitoneal injections of  $^3\text{H}$ -testosterone.

Pfaff (1976) concludes by saying that, "Taken together, this work suggests that estradiol and testosterone binding in brain, as studied autoradiographically, is one step in the physiological process by which the brain responds to these steroid hormones and controls pituitary function and mating behaviour".

Stumpf *et al.* (1976) said that "the autoradiographic data demonstrate: (1) female and male sex steroids concentrate in neurons of the nervous system in different vertebrate species; (2) in the different vertebrate species the anatomical distribution of estrogen target cells is similar and is related to phylogenetically old structures of the brain; (3) the stria terminalis appears to be the main interconnecting and integrating system of sex hormone target neurons; (4) female and male sex steroids concentrate in certain cells of the pituitary, and (5) estrogen target cells exist in the rat ependymal organs, such as the pineal, the subfornical organ, the vascular organ of the lamina terminalis, the infundibulum and the area postrema".

Scott *et al.* (1976) thought that small but physiologically important and ubiquitous bioactive molecules may be absorbed from the CSF and specialized ependyma of the median eminence transported them to the portal bed. For the proper anterior pituitary metabolism several systems may act together in an integrated way. "Direct-projecting tuberoinfundibular axons from the endocrine hypothalamus probably represent the rapid phasic neuronal input to the portal vasculature and may be superimposed over a tonic, low-grade transependymal input which may serve to control baseline levels of pituitary activity and metabolism. In view of these data specialized ependyma (tanycytes) must be regarded as an active, dynamic population of cells which may contribute significantly to alterations in the peripheral endocrine milieu".

Cardinali *et al.* (1976) concluded that marked changes have taken place in the pineal as vertebrates have evolved from amphibians to mammals. In the amphibia the pineal is a photoreceptive organ which sends nervous information to the brain via pineal nerves; the mammalian pinealocytes are neuroendocrine transducers having no direct connections to the brain and their metabolism is controlled by an indirect pathway involving the peripheral sympathetic nerves. In the avian, the pinealocytes probably acts as photoendocrine transducers by converting a photic input reaching them directly through the skull into a hormone output.

The authors further suggest that endocrine signals coming to the pinealocytes through the general circulation are active modulators of pineal function. There is a decrease in the pineal melatonin synthesis after castration in male and female rats. Physiological doses of testosterone or estradiol increase the synthesis significantly. Protein synthesis, which probably includes antigonadal peptides, is also enhanced by low doses of estradiol and testosterone in castrated rats.





Melatonin and antigonadal peptides act through the brain to depress the rate of gonadal maturation and to interfere with subsequent gonadal function and cyclicity. Therefore a negative feedback operates between the gonads and the pineal gland in mammals.

Anand Kumar *et al.* (1976) drew attention to the circumventricular ependymal derivatives integrating the nervous and endocrine systems. The tanycyte ependyma lining the anterolateral walls of the third ventricle of the hypothalamus of the Rhesus monkey have a possible role in the hypothalamic regulation of anterior pituitary function. The tanycytes form the cellular link between the third ventricle and the blood vessels of the median eminence (Anand Kumar and Knowles, 1967). Morphological changes in relation to the endogenous levels of gonadal hormones have been noted at the luminal surface of the tanycytes and a very high concentration of silver grains has been found here in autoradiographic preparations of brain from monkeys given  $^3\text{H}$ -estradiol. Estradiol has also been detected by them in the CSF. They postulated that, "the luminal surface of tanycytes might detect or absorb ovarian steroids (or their metabolites) from the CSF and that they discharge substance affecting pituitary gonadotrophins through their terminals ending on blood vessels (Knowles and Anand Kumar, 1969). Knigge and Scott (1970) have suggested that the tanycytes transfer gonadotrophin releasing hormone (GnRH) from the CSF to the portal vessels. Scott *et al.* (1976) found *positive* immunohistochemical reaction to GnRH in the tanycyte process.

In the anterior tuber cinereum of the Rhesus monkey cells with large coarse cytoplasmic granules have been noted by the authors. The contents vary according to the reproductive-endocrine status of the monkey. These cells are innervated by adrenergic nerve terminals. They are directly involved in the regulation of pituitary gonadotrophin secretion.

Anand Kumar *et al.* (1976) state that "the above findings indicate that the pineal receives both neural and hormonal inputs. These findings, viewed against the known effects of pineal principles being able to enhance or inhibit pituitary gonadotrophin secretion, and the recent finding of a high concentration of GnRH in the pineal have led to the suggestion that the pineal may constitute a second site at which the endocrine functions of the pituitary and gonads are integrated".

Regarding the book "Anatomical neuroendocrinology" by Stumpf and Grant (1975) it has been said that it provides maps and atlases of hypothalamic and extrahypothalamic sites of hormone uptake with relationship to pituitary feedback regulation, modulation of behaviour, electrical activity and actions of neurotransmitters and polypeptide messengers. Data are provided on neurotransmitter-hormone interrelationships. This book is a landmark in the history of neuroendocrinology. It proves the concept *The brain as a gland. The neuroendocrinology of the brain stem and the periventricular brain* stands in contrast





to the past era of median eminence-hypothalamic neuroendocrinology. This infundibular recess organ now appears to be but one among many similar neuroendocrine structures.

Stumpf(1975) states that "if we accept as integral parts of the brain such tissues as ependyma, choroid plexus, infundibulum-infundibular process, vascular organ of the lamina terminalis, subfornical organ, pineal complex and area postrema, then the brain, in addition to being a neuro-neuronal sensory-motor link, is a neuro-vascular and neuro-ventricular organ."

Accumulations of steroid hormone concentrating neurons have been noted in juxtaventricular areas specially at the ventral aspects and ventral extension of the ventricles. Estrogen target neurons are accumulated mainly in the preoptic hypothalamic and medial amygdaloid regions, while natural glucocorticosteroids are found mainly in the hippocamposeptal and lateral amygdaloid piriform cortical fields. Phylogenetic older parts of the pallium also contain some steroid hormone target neurons. In the mice studies, the concentration of  $^3\text{H}$ -estradiol is generally more extensive and intensive compared to the Sprague-Dawley rat. They further suggest that due to high vascularization and accumulation of estrogen concentrating cells in conjunction with the anatomical localization led them to suggest the existence of circumventricular organs in the colliculus caudalis and mammillary recess. Postulation about the existence of an organum colliculi caudalis at the rostral recess of the fourth ventricle could even be done. He further states, "In addition, the similarity of structure and the proximity of the subependymal n. triangularis septi to the organum subfornicale suggests that in the mouse and rat these structures belong together and that the n. triangularis septi form the rostroventral part of the organum subfornicale: the n. preopticus medianus is likely to be included."

Eisenfeld(1975) made schematic drawing to represent the initial steps in the interaction of estradiol with the uterus. Estradiol attaches to an 8-S binding protein in the cytoplasm. The complex crosses the nuclear membrane either before or after conformational change of the protein to 5 S. The complex attaches to acceptor proteins on chromatin and modulates RNA synthesis.

After *in vivo* administration,  $^3\text{H}$ -estradiol concentrates in the anterior pituitary, hypothalamus, and portions of the limbic system. The accumulation is highly specific for estrogens. Cytosol binding proteins with high affinity and selectivity for estrogens have been noted in the hypothalamus and pituitary. Evidences are there to suggest that the cytosol binding proteins in these sites may translocate to the nucleus after the estrogen attaches. Androgens accumulate in the hypothalamus and pituitary. There is no striking preferential accumulation of  $^3\text{H}$ -progesterone among brain regions or pituitary.  $^3\text{H}$ -corticosterone, but not  $^3\text{H}$  dexamethasone, preferentially accumulated in the hippocampus, which has the highest concentration of cytosol  $^3\text{H}$ -corticosterone binding proteins among brain regions.





The ventricular recess organs are associated with labeled neurons. There is labeling of the ependyma in different regions of the brain stem. "The present observations regarding the morphology and extensive labeling of the ependyma in the colliculus caudalis, associated with the high vascularization and neuronal labeling of adjacent structures, suggests that this is of functional significance. It is possible that this represents a hitherto undescribed circumventricular structure, the *organum colliculi caudalis*. This collicular organ at the entrance of the fourth ventricle may represent an anatomical counterpart to the organ at the exit of the fourth ventricle, the area postrema, and other *midline entrance or exit organs*, such as the vascular organ at the optic recess, the organ subfornicale between the foamina of Monroe, and the subcommissural organ at the entrance of the aqueduct".

Sheridan *et al.* (1975) studied the distribution of neurons that concentrate estradiol and testosterone or their metabolites in the brain of 2-day-old female rats by using autoradiography. These neurons were found in the preoptic region, the basal hypothalamus, and the amygdala. These studies demonstrate a common anatomical substrate upon which both estrogens and androgens, or their metabolites, may act during the *critical period* of sexual differentiation of the brain.

Sar and Stumpf (1975) studied cellular localization of progestin and estrogen in guinea pig hypothalamus by autoradiography. They injected  $^3\text{H}$ -progesterone or  $^3\text{H}$ -estradiol-17 $\beta$ , and used dry- and thaw-mount autoradiography. Progestin target neurons were present in the nucleus (n) preopticus periventricularis, n. preopticus suprachiasmaticus and n. arcuatus. In addition to these areas, estrogen target neurons have been noted in the parolfactory region, n. preopticus medialis, n. interstitialis striae terminalis, n. septi lateralis, n. paraventricularis, n. supraopticus, n. periventricularis, and in the arcuate-ventromedial hypothalamic region, including the n. arcuatus, n. ventromedialis-pars ventrolateralis, n. premammillaris, and n. magnocellularis mammillaris prelatensis. Estrogen target areas appear to be more extensive. Estrogen pretreatment increases progestin uptake. The results suggest that identical hypothalamic neurons can be acted upon by progestin and estrogen.

Martinez-Vargas *et al.* (1975) said that, "the close correspondence between the distribution of estrogen concentrating neurons in avian and mammalian brains indicates that that underlying neuronal systems have a remarkable degree of phylogenetic stability in the face of widely different evolutionary pressures".

Petrusz (1975) located the immunoreactive gonadotrophins in several neural and nonneural structures in the brain of the rat by immunoperoxidase histochemistry, using an anti-human chorionic gonadotrophin serum that cross-reacted with rat gonadotrophins.



*Gonadotrophin sites* in the rat hypothalamus are suprachiasmatic nucleus, ventromedial nucleus (ventromedial part), arcuate nucleus, periventricular nucleus and ventral premammillary nucleus. The *nonneural gonadotrophin sites* in the rat brain are the ependyma of third and lateral ventricles, hypendymal cells in median eminence, chorioid plexus of lateral ventricle and subcommissural organ.

Kozłowski *et al.* (1975) found Gn-RH in some neurons of the arcuate nucleus and fibrillar elements in the Zona externa of the median eminence of the mouse and sheep by the immunoperoxidase bridge technique. Neurophysin was detected in the supraoptic and paraventricular nuclei, in their cell processes in both the Zona externa and interna of the median eminence, and in the neural lobe. Gn-RH could not be detected in elements of the magnocellular system. Gn-RH and neurophysin staining predominated in different areas of the median eminence but both the neuropeptides were common to the Zona externa.

Kawakami and Kimura (1975) concluded that, "the brain areas involved in the estrogen-neuron system plays a fundamental role in the control of ovulation, although each part in this system is not uniformly affected by estrogen. It is apparent that the temporal and spatial characteristics of action of estrogen and progesterone determine the functional interaction between the hormones and this system".

Stumpf and Sar (1975) considered that  $^3\text{H}$ -dexamethasone is a synthetic glucocorticosteroid and autoradiographic studies with it show considerable differences when compared to  $^3\text{H}$ -corticosterone which is a natural glucocorticosteroid. In contrast to  $^3\text{H}$ -corticosterone,  $^3\text{H}$ -dexamethasone enters the brain very slowly and through the ventricular system. This is probably due to a blood-brain barrier. Apparently there is no such barrier for corticosterone.

"In the hippocampus, septum and cortex, only weak neural labeling is apparent with  $^3\text{H}$ -dexamethasone which, however, in contrast to  $^3\text{H}$ -corticosterone, shows neuronal labeling in periventricular nuclear groups in the preoptic and hypothalamic regions, and concentrates in glial cells, endothelial cells and ependymal cells throughout the brain. From these results one can conclude that the sites of action in the brain are different between corticosterone and dexamethasone. These two compounds may therefore not be used as pharmacological substitutes for each other, as is commonly done".

Rees *et al.* (1975) contrasted the localization of dexamethasone and corticosterone in the rat brain.





Autoradiography after injection of

$^3\text{H}$ -dexamethasone

- (1) A steep concentration gradient of radioactivity from the ventricular and cortical surfaces was seen 30 min after injection.
- (2) Peak accumulation of radioactivity occurred later.
- (3) Concentration was by all cell types in the brain, including choroid plexus, epithelial cells, endothelial cells, glial cells, and cells of the circumventricular organs and meninges and also by neurons.
- (4) Dexamethasone—concentrating cells were noted in most regions surrounding the lateral and third ventricles.
- (5) The nuclear concentration of dexamethasone generally was relatively weak.

$^3\text{H}$ -corticosterone

- (1) It did not show such.  
Inference = entry into the brain by different route.
- (2) Peak accumulation of radioactivity occurred earlier. Inference = rate of penetration is variable.
- (3) Concentration was primarily by neurons and weakly by choroid plexus epithelial cells.
- (4) Corticosterone—concentrating neurons were found primarily in the hippocampal formation, nuclei septi lateralis, amygdala, piriform cortex, entorhinal cortex, and suprarhinal cortex, cingulate area.
- (5) The degree of nuclear concentration of corticosterone was heterogeneous, ranging from weak in periallo-cortical cells to very strong in hippocampus.

Stumpf and Sar(1975) observed highest accumulation of radioactivity after injection of  $^{125}\text{I}$ -labeled triiodothyronine or thyroxine in the choroid plexus, the ependyma in the region of the optic recess and infundibular recess, in the infundibular process and in the pars distalis of rat's pituitary. The radioactively labeled circuminfundibular region, probably representing an area covered by ependymal tanycytes and processes of them, corresponds well to the so-called *hypophysiotrophic area*.

Hokfelt *et al.*(1975) said that, "with the indirect immunofluorescence technique, the localization of four catecholamine-synthesizing enzymes and of the luteinizing hormone releasing hormone(L R H) has been traced in the central nervous system of the rat. With antibodies to dopadecarboxylase, neurons were stained with a localization corresponding to known dopamine and 5-hydroxytryptamine systems, and in addition hitherto unidentified cells mainly in the hypothalamus, possibly containing a monoamine-like substance. Dopamine  $\beta$ -hydroxylase was found in neurons localized similarly to known noradrenaline and possibly also adrenaline systems. Phenylethanolamine-N-methyl-transferase was observed in a comparatively limited number of cell bodies in the medulla oblongata and in nerve terminals in various areas of the brain stem and the spinal





cord, probably indicating the existence of central adrenaline neurons. LRH-positive nerve endings were observed in high numbers in the external layer of the median eminence and in single axons in certain other hypothalamic and extrahypothalamic areas".

Brownstein(1975) said that histamine appears to be synthesized and stored in axons starting from nerve cell bodies in the brain stem and mesencephalon. Highest level of histamine in the hypothalamus was noted in the median eminence. In the median eminence of the rat, some or all of the histamine may be in the mast cells. He further states that the mast cell histamine is inactive from the neuroendocrine point of view. Axons terminating near these cells may lead to discharge of histamine into the portal circulation. Mast cells are not present in the parenchyma of the hypothalamus. So, the histamine that was measured in the hypothalamic nuclei may be present in the nerve endings. Highest level of histamine was found in basal and posterior areas. In this way, acetylcholine and epinephrine are also important and their distribution is reflected by the presence of their respective biosynthetic enzymes, choline acetyltransferase and phenylethanolamine-N-methyl-transferase.

### EPENDYMAL CELLS

Fully developed cells are arranged either as a simple or pseudostratified columnar ciliated epithelium. Ependymal fibers originate from the basal part of the ependymal cells. The fibers are long having repeatedly branching cell processes.

The ependymal cells of the normal ventricular system in man may be tall columnar ependymal cells with or without flagellum or cilia, or a high or low cuboidal type. Sometimes very flat ventricular lining cells are noted. This type of flat lining cells have been commonly met with by Kuhlenbeek(1970) in the ventricular border of certain specialized areas, such as area postrema, infundibular and pineal recess, supraoptic crest, subfornical organ and at the lining by tela chorioidea. Tall columnar ependymal cells without cilia and occasionally with flagellum have been noted in subcommissural organ which is situated at the basal part of the posterior commissure. In the posterior hypothalamus of Anamnia and Amniota, a thick, vascularized ependyma can be seen. This is the *ependymal organ* of Kappers-Charlton.

Kappers, Huber and Crosby(1967) stated that the non-nervous tissue within the central nervous system differentiates into ependyma, the choroid plexuses and the neuroglia. In the process of differentiation certain ectodermal supporting elements are noted to send processes from cell bodies near the ventricle to the periphery. These processes branch near the periphery and the ends anastomose or have interlacing forms to give rise to the external limiting membrane. These cells are called spongioblasts and they ultimately form the ependyma and neuroglia. They said, "It appears quite certain that the ependyme may have a secretory function, at least in certain regions. Thus, along the ventricular wall of the thalamus in certain fishes, reptiles, birds and even mammals, there are patches of



tall ependymal cells, overlying a rich capillary plexus and often giving evidence at their surfaces of an albuminous deposit. . . . . Granules can also be demonstrated in ependymal cells, particularly in developing animals. Wislocki and Putnam(1921) showed that in the area postrema fluids will penetrate from the blood vessels into the ventricle. Frederikse has demonstrated inter-cellular substance between the ependymal cells similar to that between choroid cells".

### ENZYMЕ HISTOCHEMISTRY OF EPENDYMAL CELLS

Positive reactions for A T Pase, A M Pase, T P Pase, A K, and A C have been noted in the ependymal cells. More enzyme activity is noted at the apical or luminal borders of the cells (Manocha *et al.*, 1967). Towards the border of the lumen the T P Pas-positive Golgi apparatus was found and no Golgi material was detected in the rest of the cytoplasm (Shanthaveerappa and Bourne, 1965; Shantha *et al.*, 1967). The nucleolus in these cells reacted positively for phosphatases, except for acid phosphatase.

A strong positive reaction for S D H, C Y O, L D H, G -6- P D, A D, and N A D and N A D P—linked diaphorases was noted in the ependymal cells. The reaction was in the form of diffuse fine granules. The enzymatic concentration was at the distal surface of the cells which indicated a strong oxidative metabolism. In the infundibulum low S D H activity was noted by Friede(1966). The ependymal cells of the spinal cord in the squirrel monkey showed a strong positive reaction for A P and specially in the nucleus, but negative reaction for U D P G was noted in these cells.

AChE and MAO were present in negligible amounts in the ependymal cells.

Manocha and Shantha(1969) observed that, "From the various enzyme reactions, it may be surmised that the ependymal cells are very active functional cells, with the greater part of their metabolic activity concentrated toward their apical parts. The functional significance is primarily that the concentration of the metabolic activity at the apical part of the cell is related to ciliary movements and secondarily the production or absorption of C S F".

#### *The infundibular organ of Amphioxus (Branchiostoma)*

In the anterior end of the spinal cord of *Amphioxus* there is an epichordal apical vesicle. Kuhlenbeck(1975) said that the apical vesicle can be regarded as an *archencephalon* homologous to the *embryonic prosencephalon* of Craniote vertebrates. "*Amphioxus* seems to represent an essentially spinal animal, lacking brain subdivisions comparable in detail to Craniote *medulla oblongata*, *cerebellum*, *mesencephalon*, *diencephalon* and *telencephalon*".

The cavity of the *cerebral vesicle* is lined by ciliated cells of ependymal type. In the area of the neuroporic recess a pigment spot and granulated ependyma are there. The paired *nervus apicis* is situated below the neuroporic



area and it is connected with the brain. The *pit of Kolliker* or *olfactory pit* is usually displaced towards the left. This pit is a remnant of the external neuro-pore and it may act as a chemoreceptor organ. Posteroventrally the *infundibular organ* is formed by tall columnar cells with one or two flagella per cell at their ventricular surface (fig. 1.1). A nerve comes out from the other end of the cells.

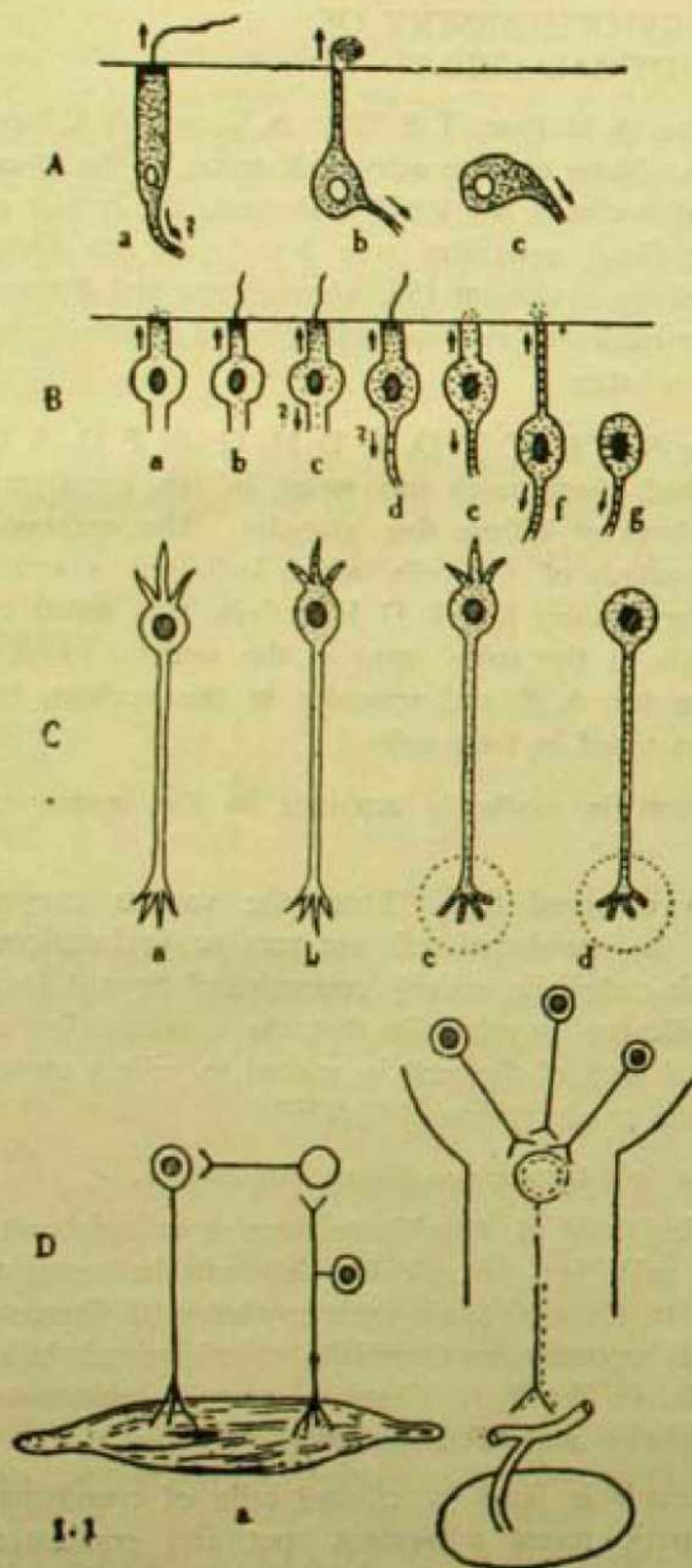


Fig. 1.1. A-D. To show phyletic origin of the neurosecretory cells of the hypothalamus. (A) After Olsson and Wingstrand (1954). Cells of the *Infundibular organ* of the *Amphioxus* (a). Bipolar ganglion cell of the nucleus praeopticus magnocellularis of the fish and amphibian (b).

(B) After Olsson (1956). One row of cells producing CHP positive substance.

(a) Juvenile subcommissural cell.

(b) *Flexural cells* (flexural organ, Olsson).

(c) Subcommissural cell.

(d) Cell of infundibular organ.

(e-g) Neurosecretory cells (Nucleus praeopticus).

(C) After Hanström (1954).

Figure of ordinary nerve cells.

(a) Ordinary nerve cell with dendrites and axon with very small quantity of noradrenalin/acetylcholine.

(b) Neurosecretory cell with neurosecretory granules or droplets in the perikaryon.

(c) Neurosecretory cell with transport of granules from cell body to the storage organ along axon.

(d) Another type without dendrites.

(D) After E. Scharrer (1952). Transformation of ordinary nerve cell.

(a) Internuncial neuron.

(b) Neurosecretory cells of the hypothalamus.

—From Diepen (1962).



These cells functionate as a ventricular sense organ. Kuhlenbeck says since it is located in a topologic neighbourhood corresponding to the mammillo-infundibular or posterior hypothalamic region of vertebrates, the infundibular organ, regardless of its dubious function, might be evaluated as kathomologous to the Craniote Vertebrate neurohypophysis or to the saccus vasculosus of Fishes or to a topologic neighbourhood providing a common configurational origin for both. A preoral pit in the roof of the oral cavity (*Hätschek's pit*), located to the right of the notochord, could represent a kathomologon of the craniote vertebrate adenohypophysis".

Olsson and Wingstrand(1955) found *Reissner's fiber* to originate from the infundibular organ and thus it "seems analogous but certainly not homologous to the *subcommissural organ* of vertebrates."

Olsson and Wingstrand(1955) noted Gomori-positive granulations in neuroectodermal cells and this is an indication of secretory activity. They thought that the hypothalamo-hypophysial neurosecretory system of the vertebrate is derived from the infundibular organ of *Amphioxus*. Kuhlenbeck(1975) says that such secretory activity can be found in different regions of the vertebrate neuraxis. He concludes that "*Amphioxus* does thus not display any configuration structurally comparable to the hypophysial complex of vertebrates, although, in a very general way, the topologic neighbourhoods corresponding to that complex are, of course, included in the overall bauplan obtaining for *Branchiostoma*".

Reissner's fiber is situated in the basal portion of the central canal of the spinal cord. A sort of filum terminale is situated at the caudal portion of the spinal cord having only ependymal wall. The Reissner's fibre terminates in this hollow filum with a *caudal mass*. Olsson(1955) did not find a posterior neuropore in the *Amphioxus* (larval and adult). In *petromyzon* the terminal sinus of the spinal cord opens through a neural pore. The caudal mass of Reissner's fibre protrudes through this into the surrounding tissues.

Reissner's fibre arises from the subcommissural organ in almost all vertebrates except in *Amphioxus* where it starts from the infundibular organ. "At embryonic stages of the Teleosts *Salmo* and *Esox* (and perhaps also at comparable stages of the Cyclostome *Petromyzon* and the Amphibian *Xenopus*), Olsson(1956) found Reissner's fibre to arise not at the subcommissural organ, as in the adult animals, but at a secretory ependyma of the mesencephalic floor, which the cited author designates as the *flexural organ*."

### *Ependymal tanocytes*

These form a functional link between the cerebrospinal fluid of the third ventricle and the primary capillary plexus of the pituitary-portal vessels. Current studies of structure and function of the tanocytes lead to the unveiling of the ways and means by which they are engaged in the hypothalamic control of the adenohypophysis.





The *typical* ependymal cells lining the upper part of the third ventricle are cuboidal, and maintain uniform size and arrangement. These cells have kinocilia and microvilli. The cells are connected by zonulae adhaerentes and zonulae occludentes. Mitochondria are located near the basal bodies of the cilia and in the area in between the nucleus and the apical ventricular surface of the cell. Endoplasmic reticulum in an organized way is not present. Ribosomes are noted in clusters and very rarely they are found near the membranes.

*Functions of typical ependymal cells—*

- (1) The cilia of the cells impart current by movement and thereby help in the rapid movement of cerebrospinal fluid.
- (2) The cells are great sources of C S F.
- (3) These cells possess the capacity to absorb. Active transport mechanism is well known by the fact that fluorescein—labeled protein can break through the ependymal barrier (Klatzo *et al.*, 1964). Brightman(1965) noted that ferritin injected into the C S F can cross the ependymal layer probably through the intercellular spaces.

In the rat these cells line the inferior wall of the third ventricle and there are multiple layers of such cells in the infundibular recess. They are irregular in shape and the size varies greatly. There are protrusions from the cells towards the ventricular cavity. Filamentous structures of variegated shape and distribution are also noted. The filaments or basal processes penetrate deeply. Bleier(1972) said that the processes extend into all areas and cell groups of the medial hypothalamus and they are most numerous in the dorsomedial, ventromedial and infundibular (arcuate) nuclei and the area of the tuber cinereum.

“The ependymal processes assume a variety of forms, lengths and calibers. They may be slender and smooth, beaded or knobby. Some divide or have branches which may be slender and perpendicular to the main process or short and tortuous; others appear to be unbranched. Many are covered either profusely with spinous processes which also vary in length and form; some are delicate and hair-like; others are knobby or bear end-bulbs. Often a number of types was seen in a single section and entering the same cell group. It was not possible to correlate any ependymal types with particular species or with any particular cell groups or area, except the median eminence”.

Different types of intricate relationships between the ependymal processes and the neurons or capillaries in the various hypothalamic cell groups were noted. Processes or side branches encircled cells and formed calyces, claws, and networks in relation to neurons. Many tanycytes send loops, end-feet, and networks around capillaries.

The proximal portion of the processes of the ependymal cells in the median eminence divides into several finer branches which commonly subdivide. The



end-branches form tuft of short processes having flattened tips which abut against the walls of fine capillaries or sinusoids situated between the infundibular part of the neurohypophysis and the adenohypophysis (a part of the hypophysial portal system). Some ependymal processes end on walls of the capillary loops which extend vertically from the interface zone into the infundibular wall.

### SPIDER CELLS IN INFUNDIBULAR NUCLEUS (BLEIER, 1972)

These cells are exclusively noted in the infundibular nucleus. With the Golgi-Cox technique these cells have a long, thick, irregularly shaped body and multiple short tortuous processes. Many short branches arise from the processes and they surround near-by neuronal cell bodies. The cells lying near the ependyma send stout, straight processes extending into the ventricular surface of the ependymal layer. Occasionally ordinary pyramidal-shaped neurons have been noted in the nucleus. Bleier(1972) stated that, "their microglial shape and their infundibular location both within the ependymal layer, adjacent to it but connected with an end-foot process, and also at some distance from the ventricle suggest a more mobile cell engaged in transport of material between the neurons and ependyma of the arcuate nucleus and the cerebrospinal fluid."

Brawer(1972) studied the fine structure of the ependymal tanycytes at the level of the arcuate nucleus. The apical membranes of these cells are characterized by a lack of cilia and by numerous microvilli. There is extensive interdigitation of the apical lateral membranes, and the junctional complexes between adjacent apical lateral membranes often lack maculae occludentes. Large Golgi apparatus is noted. The endoplasmic reticulum is principally smooth. Small paraxially oriented mitochondria have been found in the tanycyte processes. The processes also contain plenty paraxial microtubules as well as lipid droplets and accumulations of smooth endoplasmic reticulum in the form of concentric rings. Some of the tanycytes abut against the capillaries in the arcuate nucleus. These pericapillary ependymal sheaths have irregular inclusions of different densities. The ependymal tanycytes act as secretory cells engaged in hypothalamic control of the anterior pituitary gland.

Axotanic synaptic synapses have been observed by Wittkowski(1967) in the ependyma in the infundibular recess of the guinea pig. Kobayashi and Ishii(1968), and McArthur(1970) noted these synapses in the rat. Further probing is necessary to understand whether tanycytes are directly innervated. Wittkowski noted that the tanycytic processes abutting against the basement membranes of the hypophysial portal vessels had an extensive surface area and exchange of materials can take place across the area.

By injecting peroxidase into the subarachnoid space of mice or into the third ventricle of the rat, Kobayashi *et al.*(1972) found brown peroxidase reaction product in ependymal cell bodies and their basal processes in the median eminence. The reaction-positive processes terminated on capillaries of the



primary plexus or vessels surrounding the ventromedial nucleus. They suggest that the ependymal cells are involved in the adeno-hypophysial regulation by merit of their transport capacity.

Median eminence, neural lobe, pineal body, the subfornical organ, the organum vasculosum of the lamina terminalis (O.V.L.T.) and the area postrema are specialized areas which are known as circumventricular organs (CVO) of the vertebrate brain (Hofer, 1958, 1965). Special barrier properties and evidences of neuroendocrine activity have been noted in some of the CVO's. The O.V.L.T. is situated in the center of the preoptic region which controls synthesis and release of gonadotrophin-releasing hormones in medial basal hypothalamus and median eminence.

Weindl and Joynt(1972) observed that external (superficial) and internal (subependymal) capillary network are present in the O.V.L.T. of rat, rabbit, cat, squirrel monkey and Rhesus monkey as in the median eminence. The subfornical organ shows tortuous capillary loops. Special angioarchitectonic patterns are less obvious in pineal body and area postrema. Lowest degree of capillary density has been noted in the subcommissural organ. They said, "like M.E. and neural lobe, the O.V. may be regarded as a potential site of release of neurohormones. From the relatively small number of neurosecretory elements observed in the O.V. it does not appear likely that the hormone of the O.V. is released into the general circulation. Compared to the considerably larger quantities of neurosecretory material released from the neural lobe of the pituitary the dilution factor would appear too high. Although a portal relationship may be inferred from these morphological data, no substantial evidence is presently available in support for it".

The ependymal cells in M.E. and O.V. have some cytological features in common. The non-ciliated ependymal cells have long basal processes and these are rich in microfilaments and they are apposed to the peripheral basal lamina of the perivascular space. "The abundant amount of microfilaments in the long ependymal processes may be necessary to provide a mechanical skeleton. Although transport is made very suggestive by this conspicuous structural link between two fluid phases, the occurrence of similarly structured long ependymal processes in the spinal cord (Oksche, 1971) does not support the transport hypothesis. Microtubules which have been inferred as mediators of axonal transport in neural cells are not found in these ependymal processes".

Scott *et al.*(1972) investigated the mammalian median eminence. They utilized transmission and scanning electron microscopy, cytochemistry and the vascular perfusion of microfil for assessing the functional role of the numerous neuronal, ependymal, and vascular components of the median eminence of rats, cats and squirrel monkeys. The median eminence of normal squirrel monkeys had plenty of dense core vesicles (D.C.V.). Number of D.C.V. increased in rats after bilateral adrenalectomy with subsequent dexamethasone treatment.





"Intermediate range D C V, large osmiophilic neurosecretory vesicles and junctional structures between the apical plasmalemma of ventricular ependyma were selectively opacified with such reagents E - P T A and B I U L which suggests the presence of certain basic amino acid residues. The presence of dense inclusions harbored in ependymal perivascular end-feet as well as unique ultrastructural alterations of their ventricular apices had lead to discussion of their potential participation in basic neuroendocrine events".

Kobayashi(1972) studied ependymal absorption in higher vertebrates. The ependymal cells of the median eminence of the rat, mouse and quail can absorb peroxidase injected into the subarachnoidal space or the third ventricle. "The ependymal cells have a capability to absorb the ventricular fluid and send it to the terminals of their processes. From these findings it is likely that the ependymal cells of the median eminence absorb the ventricular fluid and send it to the terminals of their processes, and then they secrete substances in the fluid into the connective tissue space. In this way the substances may reach the adenohypophysis."

Rinne(1972) obtained results after bilateral adrenalectomy in rats which support the view that the aldehyde-fuchsin-staining substance and the large granular vesicles might be engaged in the neurohumoral control of corticotrophin secretion. He could not however, find any evidence for the monoamines in the tuberohypophysial system to play a role in this process. The large granular vesicles might act as storage site of C R F and might be the subcellular structures corresponding to the aldehyde-fuchsin-staining substance in the median eminence.

Witkowski and Bock(1972) said that, "The diameter of the Gomori-positive elementary granules in the zona externa under experimental conditions is, thus, seen to be extremely variable. The size of the elementary granules of the zona interna, however, is never attained. Thus, our findings support the assumption that different hormones and their carrier substances are concerned—oxytocin and vasopressin in the zona interna and the C R F in the zona externa."

Leonhardt and Eberhardt(1972) noted dye transport from the median eminence to the hypothalamic wall.

Rodriguez(1972) said that "the histological study clearly shows that most ependymal cells of the median eminence are interposed between the C S F and the portal capillaries. The ultrastructure of these cells strongly points to the possibility that they may perform a transport function. The fact that these cells may absorb exogenous peroxidase present in the C S F could be taken as an indication that transport may occur from the C S F towards the basal processes. Transport in the opposite direction cannot be excluded. The presence of (zinc iodideosmium tetroxide impregnation technique) ZIO-positive material



within tubular formations of these cells might indicate that some of the substances being transported are monoamines or related compounds."

Lofgren(1959 a, b), Leveque and Hofkin(1961), and Wittkowski(1968) noted various forms of secretory-like materials in selected regions of the third ventricle. Colloid-like substance staining faintly blue with paraldehyde-fuchsin was noted by Lofgren(1959 a). This substance was thought to be originating from inside the ependymal layer and was maximally present in those nuclei subjacent to the ependyma. Lofgren(1959 b) noted varying amounts of *foam-like* substance in the infundibular recess of the rat. This substance was, not Gomori-positive. It stained very faintly with paraldehyde-fuchsin or chromealum-haematoxylin-phloxin but stained strongly with Bodian-methods. This material is of a secretory nature and composed of thinwalled vesicles of different sizes. The vesicles are arranged in rows on the ventricular floor or piled high so that the ventricular lumen takes up honey-comb appearance. The vesicles contain amorphous granules. This material apparently came from the hypothalamic nuclei and passed through the ependyma into the infundibular recess. He found that, "In the nuclei of tuber cinereum is an abundance of extra- as well as intracellular secretory material. The accumulations of the extracellular secretory material is particularly abundant subependymally". Scheector and Weiner(1972) found that in control rats the floor of the third ventricle is smooth surfaced and there are few microvilli or other irregularities. Bleb-like protrusions are plenty in the lateral recess and the surface is more irregular. Many protruding processes at the ependymal surface of the ventricular floor are found within five minutes of intraventricular injection of epinephrine or dopamine and it looks very irregular. There are also plenty of pinocytotic vacuoles, coated vesicles and microvilli and cytoplasmic protrusions. These structures were not noted in the roof of the lateral recess or in nearby areas. These effects persisted for 15 minutes after injection of epinephrine or dopamine. The authors suggested that the ependymal layer takes part in the secretion of materials into C S F.

Intraventricular administration of dopamine in male rats gave rise to quick release of L H and F S H (Porter *et al.* 1972). A decrease in prolactin release takes place. With small doses of epinephrine or norepinephrine there was no modification in L H, F S H or prolactin release. Larger doses stimulated L H and F S H release but prolactin secretion was inhibited. Kamberi *et al.*(1970) observed inhibition of L H release after serotonin and melatonin. Kamberi *et al.*(1971) observed inhibition of F S H and stimulation in the release of prolactin. Cells which are activated by dopamine are not situated near the primary plexus of the hypophysial portal system (Porter, *et al.* 1972).

Leonardelli *et al.*(1973) could precisely locate luteinizing hormone—releasing hormone (L H—R H) by immunochemical methods. These nerve fibres were located in the median eminence of rats, mice, guinea pigs and hamsters. They terminated on the capillaries of the portal plexus and largest concentration was





noted between the premamillary recess of the third ventricle, into the dorsal labium and to each of the lateral labia.

Barry *et al.* (1973) could localize L H—R H containing perikarya in the hypothalamus of the guinea pig by immunoreactive technique. The animals were treated with colchicine. These cells were located in a vast area extending from the preoptic and septal regions to the caudal part of the tuber cinereum. Maximal distribution was found within  $\pm 1$  mm of the midline plane. These immunoreactive cells were found in the dorsal and medial septal nuclei, medial and median preoptic nuclei, anterior hypothalamic nuclei and in the premamillary region. This part corresponds to the hypophysiotrophic area and extending into septal-arcuate region.

L H—R H has been detected in ependymal cells and in axons within the median eminence, in close proximity to the basal processes of tanycytes.

Baker and Yu (1976) observed the distribution of growth hormone-release-inhibiting hormone (somatostatin) in the rat brain using the peroxidase—anti-peroxidase immunocytochemical method with antisera prepared against unconjugated, synthetic somatostatin. Low quantity of somatostatin was found in the organum vasculosum of the lamina terminalis. It was present in the full length of the median eminence and occupied the entire width between the tuberoinfundibular sulci. Most somatostatin was located in the dorsal portion of the external lamina with variation according to mediolateral position. The bodies labelled for somatostatin were most often granules. Sometimes they were in clusters which seemed to be membrane-enclosed. Some of these bodies appeared to be axons. Many of the large bodies were alongside tanycytes, but no label was distributed in tanycyte cytoplasm.

Somatostatin was highly concentrated in the proximal 1/4th of the infundibular stem and appeared in low concentration throughout the distal portion of the stem. Pars nervosa and pars intermedia was devoid of it. The distribution pattern in the median eminence was considerably different from that of gonadotrophin-releasing hormone. "Somatostatin was identified in the ventromedial and / or dorsomedial hypothalamic nuclei of only two animals. Here it was probably located in axons that terminated on neuronal cell bodies but also may have been present in a restricted portion of the perikaryonal cytoplasm."

Mitchell (1975) concluded that, "The hypothesis that tanycyte ependyma transports substances between the C S F and the hypophysial portal system and may thereby be instrumental in the regulation of the adenohypophysis is suggested by an abundance of data. Among the anatomical findings may be cited: (1) the unique morphology of tanycytes: i.e. possession of apical protrusions bathed in C S F and basal processes terminating on capillaries, (2) the restricted localization of tanycytes primarily to the infundibular recess, (3) the presense of pleomorphic granules, electrondense vesicles and other inclusions suggestive of secretory function, and finally, (4) the presence of secretory-like materials both





within the infundibular recess and between tanycytes. To this morphologic data may be added that bearing on the physiology of tanycytes, specifically: (1) the capacity to transport peroxidase and more particularly various steroids, and (2) the transitory response of apical projections to catecholamines.

Changes in the morphology of the ependymal cells in relation to changes in reproductive state have been observed in the Skunk by Hagedoorn(1965) and in the ferret by Jones(1967) and in the Rhesus monkeys by Knowles and Kumar(1969).

Knowles and Kumar(1969) studied the ependyma of the third ventricle of monkeys (both sexes and different ages) and in females at different stages of the menstrual cycle, after ovariectomy and after oestrogen treatment.

McKenna and Rosenbluth(1974) found catecholamine-containing cells bordering the infundibular recess of the toad hypothalamus. These cells could discharge catecholamines into the infundibular recess and thus effect the release of L R F from axons in the median eminence. From the evidences put forward it seems that the cells are sensory. Their ideal position allows them to respond to hormones in the ventricle and thus they are receptor cells which are able to relay information about the C S F to the neurons where they form synaptic contacts. "Specifically, their function could be to detect changes in ventricular hormone levels and in turn stimulate either directly or indirectly L R F-producing cells through their somato-dendritic contacts. The axons innervating them could serve to modulate the sensitivity of the cells to a degree that depends on the stage of the reproductive cycle".

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Klara and Brizzee(1977) studied the area postrema (AP), a circumventricular organ (CVO) of the cat by scanning (SEM) and transmission electron microscopy (TEM). The specialized CVO regions lack a typical blood-brain barrier. The barrier protects the CNS from many foreign substances. In SEM preparations the boundary of the A P was sharply differentiated by the absence of kinocilia. There were plenty microvilli and they seemed to be concentrated at the junction between ependymal cells giving rise to a polygonal pattern superimposed on cell boundaries. Some cell processes were present on the AP surface. No supraependymal cells bodies could be seen over the AP proper. In TEM preparations the AP was characterized by blood vessels with distinct perivascular spaces. Fibroblasts and collagen were found within the spaces and the spaces were limited by basal laminae. Capillary endothelial cells were typically fenestrated and contained numerous pinocytotic vesicles. Bulbous endings of attenuated cellular processes terminated on the external basal laminae of AP vasculature. Some of these endings could be traced to the cells covering the ventricular surface of the AP. These cells have several features of ependymal cells (*tanycytes*). There are groups of small neurons. Axodendritic variety of synapses could be seen and the ending contained both dense cored and clear cored vesicles. Myelinated and unmyelinated axons were also noted. AP has

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got the chemoreceptive function. The authors considered the possibility of functions in addition to emetic chemoreception. Other functions of AP are as follows.

- (a) It may have a secretory or blood flow control function
- (b) AP was the central site of action for angiotensin.
- (c) AP may act as a sensor for monitoring the serotonin (5-HT) system.

"the possibility that the AP may monitor 5-HT produced by the supra- and subependymal 5-HT plexus becomes increasingly interesting with the recent demonstration of horseradish peroxidase penetration into the CNS areas around the circumventricular organs."

Bleier(1977) studied the ultrastructural features of the ependyma and supraependymal cells of hypothalamic third ventricle of the mouse in the region medial to the dorsomedial and ventromedial nuclei and dorsal part of infundibular nucleus. Animals in different phases of the estrus and reproductive cycle were used. Different ependymal cells have different functions and the hypothalamic ependymal layer as a whole reflects in its morphology the changing metabolic states of the animal. The features suggested a dynamic relationship between ependyma and csf. Relations between tanycyte processes and neuropil have also been described. Many of the supraependymal cells are macrophages. In some cells there were bizarre mitochondrial profiles associated with Golgi, endoplasmic reticulum, lysosomes and lipid droplets (GEROL and LERL).

"It is suggested that phagocytic supraependymal cells are engaged in processing the debris that results from changing metabolic activities, including secretion and exocytosis, blebbing and necrosis, of ependymal cells and also in the ingestion of foreign particles invading csf or ependyma".

DATA ENTERED



## CHAPTER 2

### DEVELOPMENT OF THE PITUITARY PORTAL VESSELS AND CELLULAR DIFFERENTIATION OF THE ADENOHYPOPHYSIS.

Glydon(1957) described the development of the hypothalamo-hypophysial vascular system in the rat. Enemar(1961) described them in the mouse. Terneby(1972) described the same in the rabbit. Halasz *et al.*(1972) described the ontogenesis of the neurovascular link between the hypothalamus and the anterior pituitary in the rat. Ultrastructure of the median eminence of neonatal and adult rats was studied by Monroe *et al.*(1972). Dearden and Holmes(1976) studied the cyto-differentiation and portal vascular development in the mouse adenohypophysis.

Enemar(1961) noted in the mouse that the superficial plexus appeared 15-16 days postcoitum (p.c.). At 16 days p.c. the short portal vessels appeared and there was internal vascularization of the pars distalis. Long portal vessels and superior hypophysial artery appeared between 18-19 days p.c. Inferior hypophysial artery appeared at 1 day postnatum (p.n.). At the same period deep capillary loops also appeared. The tangential plexus was noted at 4-7 days p.n. The superficial capillary loops appeared at 9-21 days p.n.

Glydon(1957) observed the superficial plexus at 19 days p.c., short portal vessels at 21 days p.c., internal vascularization of the pars distalis at 16 days p.c., and the deep capillary loops at 5 days p.n. in the rat.

Terneby(1972) noted the time of appearance of the following in the rabbit as follows: The superficial plexus (19 days p.c.), short portal vessels (21 days p.c.), internal vascularization of the pars distalis (16-17 days p.c.), long portal vessels (22-23 days p.c.), superior hypophysial artery (17-18 days p.c.), inferior hypophysial artery (21 days p.c.), deep capillary loops (22 days p.c.), and superficial capillary loops (28 days p.c.).

According to Dearden and Holmes(1976) the time of appearance of the structures in the mouse are as follows: the superficial plexus (16 days p.c.), short portal vessels (17 days p.c.), internal vascularization of the pars distalis (17 days p.c.), superior hypophysial artery (19 days p.c.), long portal vessels (1 day p.n.), inferior hypophysial artery (1 day p.n.), deep capillary loops (1-2 days p.n.), tangential plexus (6 days p.n.) and superficial capillary loops (14-21 days p.n.).



Dearden and Holmes(1976) described the adult hypothalamohypophysial vascular complex of the mouse. It consists of a network of capillaries situated on the surface of the median eminence and the caudal infundibular sulcus. The superficial and deep capillary loops start from the capillary network. The superficial loops enter into the external layer of the median eminence and come back to the superficial plexus. The deep capillary loops enter into the intermediate layer and have anastomoses with nearby hypothalamic vessels. They form a subependymal or tangential plexus and then drain into the superficial plexus. The *primary plexus* is formed by (1) superficial plexus (2) superficial capillary loops and (3) deep capillary loops. The oral part of the primary plexus is situated on the median eminence and the aboral part is located in the caudal infundibular sulcus. The feeding arteries of the superficial plexus are : two superior hypophysial arteries which arise from the internal carotid artery and distribute to the oral part of the primary plexus; two arterial branches start from the posterior infundibular artery and supply the aboral plexus; one branch from the posterior communicating artery supplies the caudal part of the aboral plexus. There are about fifteen short portal vessels which supply the pars distalis and they come from the caudal part of the oral plexus and aboral plexus. Five long portal vessels start from the anterior oral plexus. Direct arterial supply from the branches of superior and inferior hypophysial arteries to the pars distalis has been observed by the authors. Venous drainage from the lateral side of the pars distalis is by two or three small veins into the cavernous sinus and the postero-inferior part drains into the inferior hypophysial vein.

The authors found three groups of PAS positive cells in the pars distalis of the mouse. The distribution pattern is similar to that noted by Purves and Griesbach (1951) in the rat. The round cells are mainly LH gonadotrophs. FSH gonadotrophs are oval cells. Polygonal cells are mainly thyrotrophs. The cytodifferentiation of these cells depends on the internal vascularization of the pars distalis. Differentiation of PAS positive cells occurred from chromophobic cells. Ultrastructurally granulated cells were noted at 16 days p.c. and they could be identified as FSH and LH gonadotrophs, thyrotrophs and corticotrophs at 17 days p.c. There is an increase in the release of FSH, LH, TSH and ACTH at 17-18 days p.c. and a decrease on 19 days p.c. to 1 day p.n. Vacuolated somatotrophs were noted in peripheral part of the pars distalis at 19 days p.c. to 1 day p.n. In the central part of the pars distalis, during the same period there were many somatotrophs without much vacuolation.

The authors further found that the number of secretory cells increased during 1-10 days p.n. with a diminution in the number of chromophobes. This proves that the secretory cells differentiate from chromophobes.

The pars distalis is under the hypothalamic control during the late foetal and earliest postnatal period.

Halasz *et al.*(1972) observed that the structure of the surface zone of the adult median eminence is obtained in foetuses of 18 days of age. Morphologi-



cally, the median eminence of a new born rat is almost identical with the adult. Kobayashi *et al.*(1968) studied the ultrastructure of the developing median eminence in rats. Halasz *et al.*(1972) noted the differentiation of the anterior lobe cells in the rat to occur after the development of the hypothalamo-hypophysial connections. The foetal pituitary cells of a 16 or 17 day-old foetus is undifferentiated ultrastructurally. From the 18th day of gestation the agranular cells start granulating. The developmental orientation of these cells cannot be identified. The granulated cells increase in number with the increase of age. At 22 days of gestation the appearance of the pituitary cells is reminiscent of the adult. Yoshimura *et al.*(1970) had similar observations.

Monroe *et al.*(1972) while studying ultrastructure of the median eminence of neonatal and adult rats observed that the contact between primary capillaries and the endings of tubero- infundibular axons in the median eminence was essentially obtained in the newborn rat and this neurohaemal contact was seen to exist along the surface of the median eminence at birth. Before the establishment of penetrating capillary loops this contact is formed. The neonatal median eminence is structurally immature. The neural layer is practically avascular except the surface. Therefore the axonal contacts which are small in number, are only noted in relation to superficial primary capillaries. The palisade zone opposed to the pericapillary area has no store of dense core vesicles which characterize this zone in adults. Mature pattern of ependymal—axonal relations has not yet achieved. They further state, "Therefore, while the structure of the median eminence of the neonatal rat provides the basis for some hypothalamic influences to be conveyed to the pars distalis, further morphological maturation may well be required before the median eminence can play its full role in neuroendocrine events."

#### *Hypothalamohypophysial portal vessels in different animals*

Different ways by which pituitary portal vessels have been studied are as follows :—

- (a) Dissection and the study of serial sections of human specimens (Popa and Fielding, 1930).
- (b) Serial sections of the median eminence and pituitary region after intravascular injections of india ink (Wislocki and King(1936) in rabbit, cat and monkey; Green(1951) in 76 vertebrate species).
- (c) Human and sheep median eminence primary plexus has been examined by light microscope after neoprene latex injection for clarification of the vascular organization (Xuereb *et al.*(1954); Daniel and Prichard, 1957).
- (d) Angioarchitecture of the median eminence has been studied by light microscopic examination of india ink injected whole-mounts with the clearing technique of Spalteholz (Duvernoy *et al.*, 1971; Duvernoy and Koritke, 1964; Duvernoy, 1972).



- (e) Vascular casts of injected specimens have been studied with the scanning electron microscope to have a 3-dimensional concept of the extent and topography of the primary plexus and also to have a good depth of field and resolution (Page and Munger, 1975; Lametschwandner and Simonsberger, 1975; and Page *et al.*, 1976).

Page *et al.* (1976) studied the pituitary vascular casts by scanning microscopy in rabbits. Page and Bargland (1977) prepared vascular casts in the mouse, rat, dog, sheep and monkey and examined them under the scanning electron microscope.

It was observed that a common vascular bed was shared by the infundibulum (median eminence), infundibular stem and infundibular process (neural lobe) when the pituitary-median eminence cast was viewed from above.

In the rabbit the hypothalamus is situated above the posterior cerebral arteries and hypophysis is situated below.

The median eminence of the rabbit is supplied by superior hypophysial arteries which arise from the paired internal carotids, the anterior cerebral and the posterior cerebral arteries. The median eminence is encircled by the superior hypophysial arteries and they approach it from above. In smaller branches they enter into the depth of the hypothalamus and join the capillary plexus of the median eminence from within. A series of parallel ascending vessels comprise the vascularity of the hypothalamus.

#### *Infundibular stem*

The anterior hypophysial artery starts from the vertical segment of the internal carotid artery proximal to its bifurcation. It proceeds backwards over the lateral surface of the pars distalis but does not supply the glandular pituitary. A branch from the anterior hypophysial artery enters the cleft between the zona tuberalis and infundibular stem at the level of the latter one.

The *infundibular process* is supplied by the paired inferior hypophysial arteries which arise from the horizontal portion of each external carotid artery. Branches of each inferior hypophysial artery proceed in a dorsomedial direction and after anastomosis they form a ring about the neurohypophysis. A branch may penetrate the dorsal part of the infundibular stem. Branches of the anterior hypophysial artery may also contribute to the formation of the aforementioned arterial ring.

The vessels of the floor of the median eminence are capillary loops having a lumen diameter of 80  $\mu$ . A reticular network of small capillaries having a lumen diameter of 10  $\mu$  is seen in the walls of the median eminence. These correspond to the *mantle plexus* of Romeis or the *surface network* of Duvernoy.

The *external plexus* is formed by vessels from the circle of Willis. These



capillaries drain into long portal vessels which descend on the surface of the zona tuberalis and then break up into secondary plexus within the pars distalis. The external plexus extends from the walls to the floor of the infundibulum (median eminence). In the floor of the infundibulum the external plexus is situated between the internal plexus of capillary loops above and vessels of the zona tuberalis below. It is not superficial in this situation. The external plexus does not get a direct artery supply within the floor. This is achieved through its continuity with the external plexus of the walls having its artery supply at the rim of the infundibulum. Additional system of confluent capillaries extends between the median eminence and the glandular pituitary. Posteriorly, the external plexus forms portal vessels which enter the pars distalis after bridging the cleft in the adenohypophysis below.

The *internal plexus* correspond to the *deep network* of Duvernoy. In the anterior and lateral walls *vascular coils* have been noted. There is a central-core vessel upon which there are coils of smaller vessels. In some cases these *spiralling vessels* join the central core vessel at the top. They start from capillaries of the external plexus. The central core vessel enters into a large portal vein.

"In the floor, anterior to the underlying vascular cleft, *vascular spikes*, rather than coils, form the internal plexus. Such spikes arise from the external plexus, ascend for a distance of about 200  $\mu$ , then turn, and retrace their course back to the external plexus of the floor. In some instances, complex arborizations occur at the apex, whereas in others the apex consists of a hairpin turn. The two limbs of a spike are of equal luminal diameter. Shunts may be present between the two limbs". The internal plexus has a broad vascular surface which appose the third ventricle within the floor of the median eminence. Direct arterial supply has not been noted by the authors to the vascular coils or spikes of the internal plexus. Instead, the supply is from the external plexus.

A confluent capillary network is noted supplying the infundibulum, infundibular stem and infundibular process. Continuity has been noted in between the capillaries of the posterior infundibular wall and the infundibular stem. There is an *avascular cleft* which is bounded anteriorly by zona tuberalis, posteriorly by pars distalis, and superiorly by vessels of posterior infundibular floor. The primary plexus of the median eminence can be divided into external and internal plexus anterior to the avascular cleft. But it is difficult to get such a division posterior to the cleft. Vessels of the walls join with the vessels of the floor in the posterior median eminence and are continuous with vessels of the infundibular stem.

Posteriorly the network of the infundibular stem enters into the *infundibular process*. "The triangular avascular cleft underlying the posterior portion of the infundibular floor extends beneath the infundibular stem and process and is bridged by plenty short portal vessels."



*Venous trunks* receive blood from infundibular process and pars distalis and drain into sinuses about the posterior part of the pituitary.

Arterial supply to the rabbit pituitary is only to the neurohypophysis. A direct supply to the adenohypophysis was not found by the authors.

Flow of blood may be from the median eminence towards the infundibular process (*anterograde*) or from the infundibular process towards the median eminence (*retrograde*). Török's reversal of flow could occur within the neurohypophysis as there is a vascular route by which neurohormones released in the infundibular stem and process could enter into the long portal system and thus into the pars distalis. The short portal system could provide the second route. The authors state that, "Thus the entire neurohypophysial capillary bed could serve as the final common pathway to the glandular pituitary."

Page and Bergland (1977) studied vascular casts of the pituitary -median eminence complex of mice, rats, dogs, sheep and monkeys with the scanning electron microscope. Microfil-injected specimens of the rabbit and monkey pituitary -median eminence complex were examined by light microscope after intracranial internal carotid artery occlusion. A common neurohypophysial capillary network, in each species was found uniting the median eminence, the infundibular stem and the infundibular process. This capillary network is supplied from above by superior hypophysial arteries and from below by inferior hypophysial arteries. In some species, an artery to the infundibular stem was found.

Flow through superior hypophysial arteries did not occur when the intracranial internal carotid arteries were occluded. Yet the whole of the neurohypophysial capillary bed filled upon injection with Microfil. These observations suggest, "the concept of a restricted artery supply to the median eminence with drainage to the underlying adenohypophysis on one hand and to the infundibular process with drainage to the systemic circulation on the other must be modified and that blood flow within the neurohypophysial capillary bed (between infundibular process and median eminence) occurs."

#### *Mouse and rat*

The capillary network was reticular and it lay exposed on the dorsum of the cast. An avascular zone, traversed by short portal vessels, separated vessels of infundibular stem and process from those of adjacent pars distalis.

#### *Dog*

The neurohypophysial capillary network was not exposed on the dorsum of the pituitary median eminence vascular cast. Superior hypophysial arteries started from the internal carotid arteries. Inferior hypophysial arteries started from the infraclinoid internal carotid arteries coursed through the cavernous sinus before arborizing within the neural lobe.



*Monkey*

The reticular neurohypophysial capillary bed was common to the median eminence, infundibular stem and infundibular process. This was exposed on the surface of the cast. Superior and inferior hypophysial arteries supplied this capillary bed from above and below. Infundibular stem artery was inconsistently noted.

*Sheep*

The artery to the infundibular stem was found consistently. It started from the carotid rete within the cavernous sinus similar to the inferior hypophysial arteries. The superior hypophysial arteries started from the reconstituted carotid arteries after they had entered the cranial vault.

Filling of the *rabbit* pars distalis was markedly reduced after intracranial occlusion of both internal carotid arteries proximal to the starting of the superior hypophysial arteries. Some filling of microfil within the median eminence was found as had filling along the dorsum of the specimen in the neurohypophysial capillary network.

*Monkey*

Intracarotid injection of microfil showed good filling of the pituitary median eminence complex. Whole of the neurohypophysial capillary bed filled up when intracranial carotid occlusion was done. There was scanty filling of the pars distalis. Filling of the infundibular process, stem, median eminence and some long portal vessels also occurred.

An avascular cleft separates the reticular neurohypophysial capillary bed within the infundibular stem and process from the nearby, linear adenohypophysial capillary bed. This cleft is noted in the rabbit, mouse, rat and dog and it is bridged by short portal vessels. It is the site of pars intermedia.

From the above it is evident that the confluent neurohypophysial capillary bed received arterial blood supply from different sites and thus flow of blood may be variable and the authors state that, "Blood flow from the infundibular stem towards the infundibulum may occur in living animals. Changes in vascular dynamics of venous drainage of, or arterial supply to, the neurohypophysial capillary bed may well alter the direction of blood flow within it. The major routes of egress from the neurohypophysial capillary bed is to the cavernous sinus in man, rat, mouse, dog, sheep and monkey (Berghand and Page, 1977). Venous pressure within the cavernous sinus varies with the physiologic state of the animal. Elevation of this pressure (as with a Valsalva manoeuvre) may impede venous outflow from the infundibular process and favour flow toward the median eminence. Elevation of systemic arterial pressure with an increase in pulse pressure will also elevate pressure within the cavernous sinus and will tend to impede venous outflow from the pituitary gland. Such a circumstance will



favour flow within the neurohypophysial capillary bed toward the infundibulum where blood may exit via capillary connections to the hypothalamus (Bergland and Page, 1977) or via portal vessels and capillary connections to the adenohypophysis."

Alterations in the direction of blood flow within the reticular capillary network may also take place due to—

- (a) differences in arterial perfusion pressure between several sites of arterial supply to the capillary bed.
- (b) changes in physical and chemical environment.
- (c) "The carotid siphon effectively elongates the distance between the origin of the inferior and superior hypophysial arteries, suggesting that the arterial pulse wave will arrive at the inferior hypophysial arteries prior to its arrival at the superior hypophysial arteries. Such observations suggest that differences in the rate of flow or perfusion pressure may exist *in vivo* between sites of arterial supply to the neurohypophysial capillary bed."

The authors suggest that reversal of flow does occur *in vivo* "and that blood flow within the neurohypophysial capillary bed from infundibular process to median eminence provides a means to deliver neurohormones to the glandular pituitary and to the brain." Page and Bergland (1977) described the pituitary vasculature wherein they have stated that the venous channels draining the adenohypophysis are less, whereas those of the neurohypophysis are plenty. A reversal of blood flow may take place within the short portal system between the adenohypophysis and the neurohypophysis. In this way they could help the venous drainage of the adenohypophysis. Adenohypophysial hormones could reach the neurohypophysial capillary bed before emptying into systemic veins and thus a *short feedback loop* may operate.

The authors conclude that "the neurohypophysis is more than a transducer converting electrical signals to chemical messengers. Its capillary bed may actively and selectively determine the destination of both hypothalamic and pituitary secretions, conveying some to the glandular pituitary, others to distal target organs and yet others to the brain."

Oliver *et al.* (1977) investigated the retrograde transport of pituitary hormones in the pituitary stalk vasculature in anaesthetised male rats in which the pituitary gland was intact and in animals in which the anterior pituitary, posterior pituitary, or entire pituitary gland had been removed 30 to 60 minutes before use. The duration of collection of blood was 1.5 to 2 hrs. by free flow from a single long portal vessel through a microcannula with its tip pointed toward the hypothalamus. Arterial blood sample was also collected. By radioimmunoassay the authors observed that the concentrations (ng/ml) of LH, TSH, prolactin, ACTH,  $\alpha$ -MSH and vasopressin in portal plasma in rats with intact pituitary were 70 to 1000.



times that noted in the arterial plasma. In anterior lobectomized rats there was great depression in the concentrations of LH, TSH, prolactin and ACTH.  $\alpha$ -MSH was moderately decreased. The concentration of vasopressin was unaffected. After posterior lobectomy there was no change in LH and TSH but a marked decrease was noted in prolactin, ACTH,  $\alpha$ -MSH and vasopressin. All the hormones in hypophysectomized animals were greatly reduced.

The authors postulated the hypothesis

- (a) that retrograde transport of pituitary hormones occur in certain vascular channels of the pituitary stalk toward the hypothalamus
- and (b) that pituitary hormones in the retrograde vasculature can reach blood in the long portal vessels going to the anterior pituitary, possibly by diffusion involving a countercurrent mechanism.

Arterial blood is delivered to the ventromedial hypothalamus by the superior hypophysial arteries, to the pituitary stalk and pars nervosa by one and sometimes two peduncular arteries, and to the pars nervosa by the inferior hypophysial arteries. The long portal vessels supply blood to the pars distalis. Venous drainage is into the sinus around the gland. A vascular pathway for retrograde blood flow exists. In either the pituitary stalk or ventromedial hypothalamus or in both regions, hormones passing in a retrograde direction in the stalk enter the blood in the long portal vessels. The authors could not be certain whether this retrograde passage was effected by diffusion between capillaries, through direct vascular connections or by both processes.

Duvernoy (1972) studied the vascular architecture of the median eminence in numerous species including birds, mammals and man. The following review is from Duvernoy's description in 1972.

The median eminence is vascularized by the dense capillary network of the primary plexus. The primary plexus is formed by two intricately linked vascular systems: the *surface network* and the *deep network*. The surface network is the *Mantel-plexus* of Romeis (1940) and covers the surface of the median eminence. It is covered by the pars tuberalis. The *surface network* is made up of capillaries with a dense but irregular mesh-like structure where arterioles supplying the network and portal vessels drainage from this network are also found. Some of the meshes enter a little into the palisade layer of the median eminence and form the short capillary loops (Engelhardt, 1956), and they look like an inverted U (Akmayev, 1971). The loops are plenty, in the rostral zone but less frequent in the caudal zone of the median eminence. The tubero-hypophysial nsm enters into the anterior lobe through the surface network.

The *deep network* (fig. 2.1.) is found in the whole of the median eminence and the infundibular stem. The deep network can be divided into 1) *long capillary loops*, and 2) *subependymal network*. The afferent limb of the long capillary loop arises from the arterioles situated in the surface network. It is



of small diameter. The afferent limb enters deep into the median eminence and crosses the palisade, reticular, and fibre layers. It increases in size as it ascends and supplies the top part of the loop which is situated in the subependymal layer. The efferent branch starts from the top of the loop and crosses the median eminence to reach the surface. This branch is often a satellite of the afferent branch with which it forms the foot of the capillary loop. Oftenly the efferent branch is very large and continues as a wide portal vessel. In some species the efferent branches of the long capillary loops appear as part of the surface network.

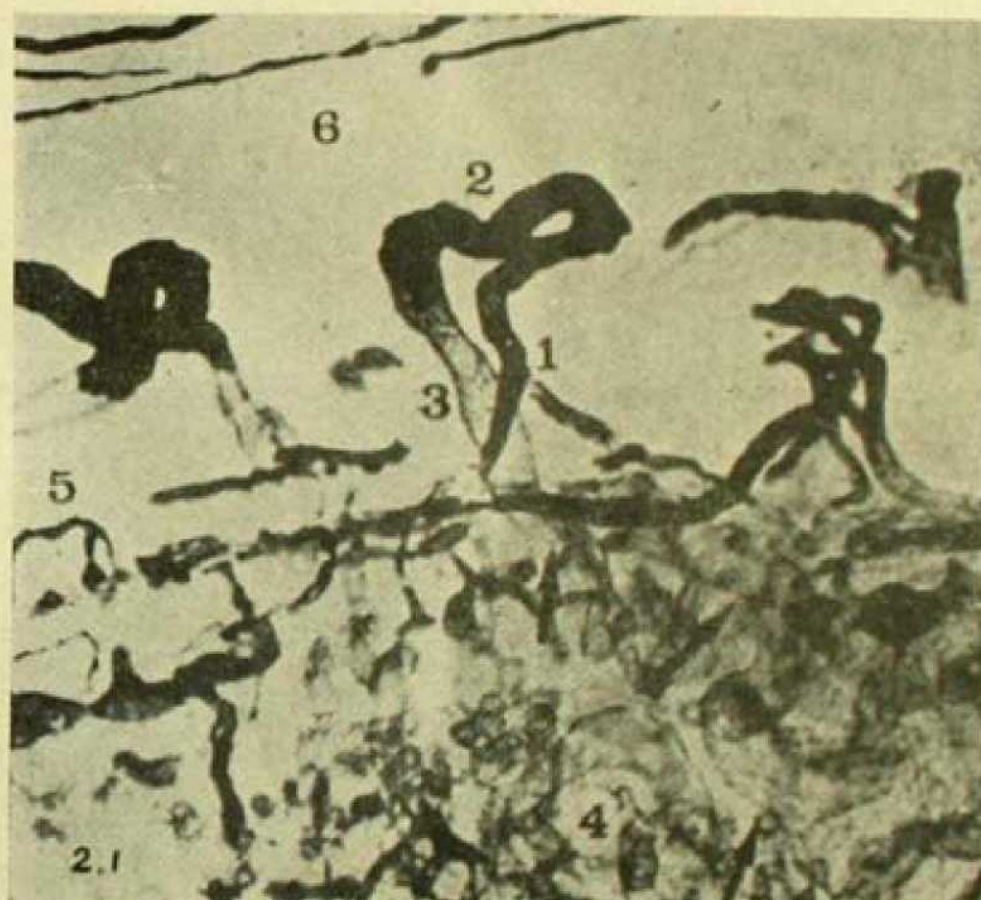


Fig. 2.1. Sagittal section of the hypophysis (dog).

- (1) Long capillary loop with narrow afferent branch.
- (2) Top of the loop situated near the ependyma.
- (3) Thick efferent branch which can be followed (arrowed) into the anterior lobe (4).
- (5) Short capillary loop stemming from the surface network.
- (6) Hypophysial recess.

—From Duvernoy (1972).

Complex arrangements are also met with. Branches form a shunt between the ascending and the descending limbs and the capillary loop may form a vascular tuft or scrawl as is noted in primates and man (Fumagalli's *Gomitoli* or *spikes* of Xuereb *et al.*, 1954). The long capillary loops are always present in mammals but are rare in birds. The density of the subependymal network is present in both.



The density of the *subependymal network* increases towards the edge of the median eminence. In this place this network has connections with the tuberal vessels.

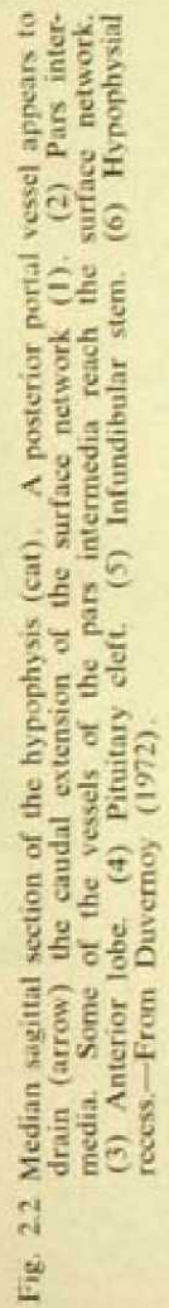
The primary plexus drains into the secondary capillary net situated in the adenohypophysis. In mammals four main groups of portal vessels have been found. They are : anterior, posterior and two lateral portal vessels (Adam *et al.*, 1969). In the fox Duvernoy (1972) found the drainage of a complex capillary loop of the median eminence by a large portal vessel which passes through the anterior lobe and reaches the caudal end where it drains into the perihypophyseal veins. These portal vessels have little branching.

The subependymal network and the top of the long capillary loops almost reach the cavity of the ventricle (Duvernoy and Koritke, 1968). Covering these areas the ependymal barrier is thin or deficient. Ependymal cells are grouped around the subependymal vascular network. The cells have also connections with the surface network. Similar neurovascular pattern has also been observed in the vascular organ of the lamina terminalis (OVLT), the subfornical organ and the area postrema. Thus the primary plexus has relationship with the ventricular fluid in the cavity of the third ventricle. The haematoencephalic barrier is permeable in the wall of the circumventricular vessels and in the wall of the vessels of the median eminence.

The primary plexus has anastomosis with *vessels of the posterior lobe* and the *hypothalamus*. The mantelplexus can be traced along the infundibular stem to the posterior lobe. This is situated in between the neural lobe and the intermediate lobe (fig. 2.2.). Some substances of the neural and intermediate lobes may reach the pars distalis by this type of anastomosis.

The primary plexus has anastomosis with the vessels of the nearby tuber (fig. 2.3.). The anastomoses although found in all the species, nevertheless, are different in nature. In *front and along both sides*, hypothalamic arterioles which reach the deep network are found, as are arterioles which pass through the median eminence and, in fact, reach the tuber. In man some veinules from the primary plexus join the hypothalamic veins (fig. 2.4.). The functions of the ascending and descending connections are difficult to find out. A supplementary portal system joining the tuber to the median eminence is not formed thereby. In the *posterior part* a vascular formation occupies the posterior tuber in man. It is very similar to the primary plexus in many respects. This network is drained towards the hypophysis by some portal vessels. The network consists of the *surface network* and the *deep network* (fig. 2.5.). One or two long portal vessels of large diameter proceed towards the posterior part of the hypophyseal stalk in man. Drainage is also towards the basilar veins. The *deep network* consists of voluminous twisted capillaries. They are subependymal in position and line the pars caudalis tuberis. The anterior capillaries drain exclusively into the portal system. Most of them drain into the portal system and towards the lateral hypothalamic veins. The capillaries in the caudal part







of the postinfundibular eminence near the mamillary bodies are exclusively towards the hypothalamus and are independent of the portal system. Duvernoy (1972) thought that, "If it is proven that some tuberal nuclei (such as the infundibular nucleus) are vascularized by this network, it is reasonable to



Fig. 2.3. Sagittal section of the anterior median eminence (dog).

(1) Median eminence. (2) Tuber. (3) Pars tuberalis. (4) Pars distalis. (5) Hypophysial recess. (6) Origin of an arteriole branch which passes through the tuber and reaches the subependymal network (arrows) (descending tuberal connections).  
—From Duvernoy (1972).

postulate that neurosecretory substances secreted in this region reach the glandular cells of the anterior lobe directly via the portal vessels. It must, however, be noted that the different aspects of vascularization of the posterior tuber are not always so clearcut in all species."



Topographical division of the portal vessels which drain certain areas of the median eminence towards certain region of the adenohypophysis is a probability.

Porter *et al.* (1977) reviewed the hypothalamic-pituitary interaction. They injected a solution of dye, lissamine green into a peripheral artery and noted the direction from which the stalk vessels filled. The small vessels in the stalk

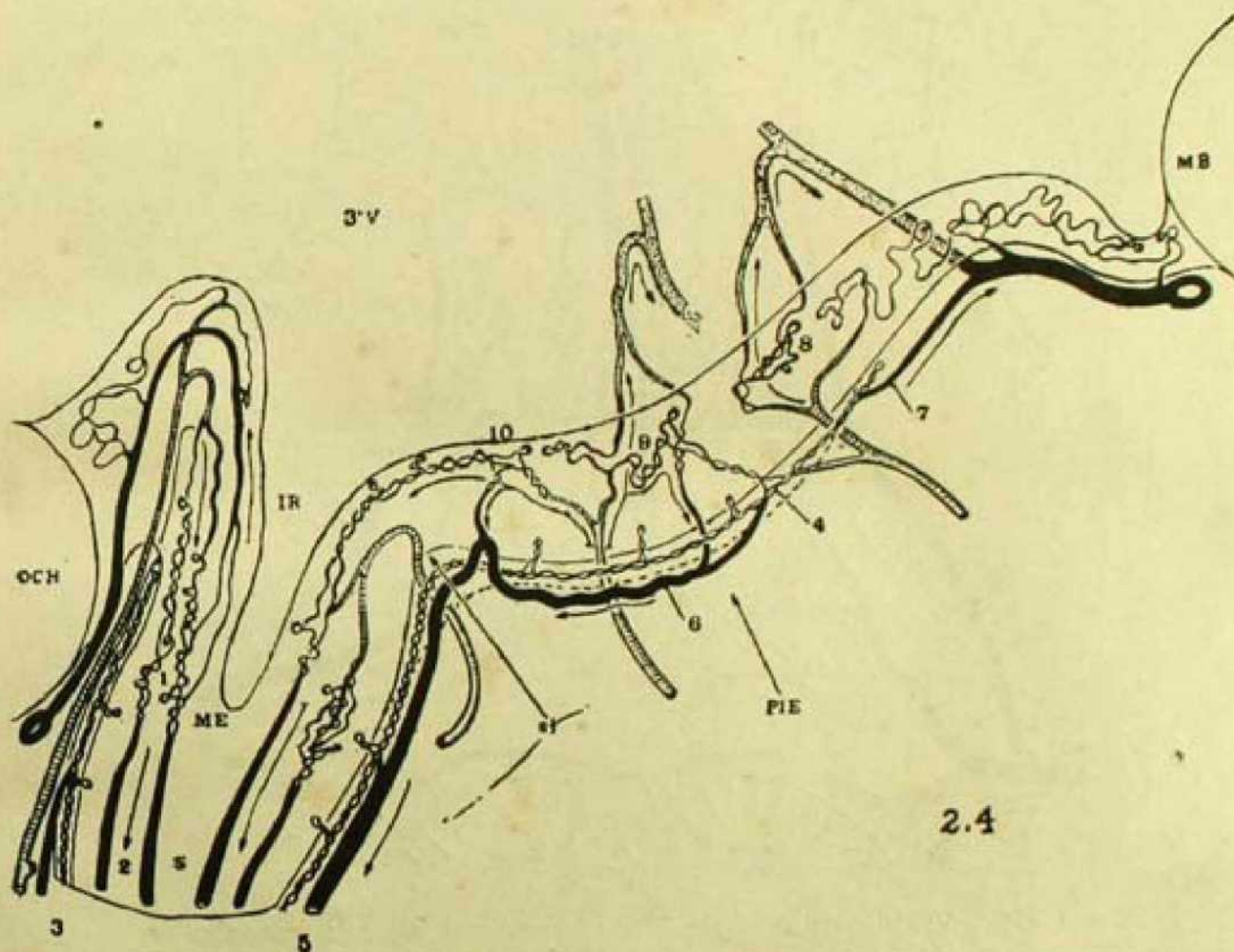


Fig. 2.4. Man. Drawing based on a median sagittal section of the floor of the third ventricle. The following elements are shown (from left to right): OCH=Optic chiasm, ME=median eminence, IR=infundibular recess. S=stalk, PIE=post-infundibular eminence (posterior tuber) separated from the median eminence by the sulcus infundibularis (SI). MB=mammillary body, 3eV=third ventricle. Vascular tuberohypophysial connections:

(a) Anterolateral connections. 1=Capillary tufts which belong to the deep network of the primary plexus and which are supplied by the tuberal arterioles (downward pointing arrow). These tuft have some veinules which join the tuberal veins (upward-pointing arrow). 2 = Portal vessels. 3 = Surface network and its drainage.

(b) Posterior connections. 4 = Superficial network lining the PIE. It continues towards the superficial network of the primary plexus (5). 6 = Portal vessel. 7 = Drainage of the surface network by a tuberal vein. 8 = Deep network which is exclusively drained by veins (arrows). 9 = Deep network with a mixed drainage (towards the hypophysis and towards the tuber). 10 = Deep network which is exclusively drained towards the hypophysis (arrow). —From Duvernoy (1972).



filled from the lower stalk upward. In the long portal vessels the filling was downward starting from the median eminence. As anterior and posterior pituitary hormones were present in high concentrations in portal vessel plasma, the authors concluded that hormone exchange happens in the pituitary stalk vessels. Through this system posterior pituitary hormones are transported to

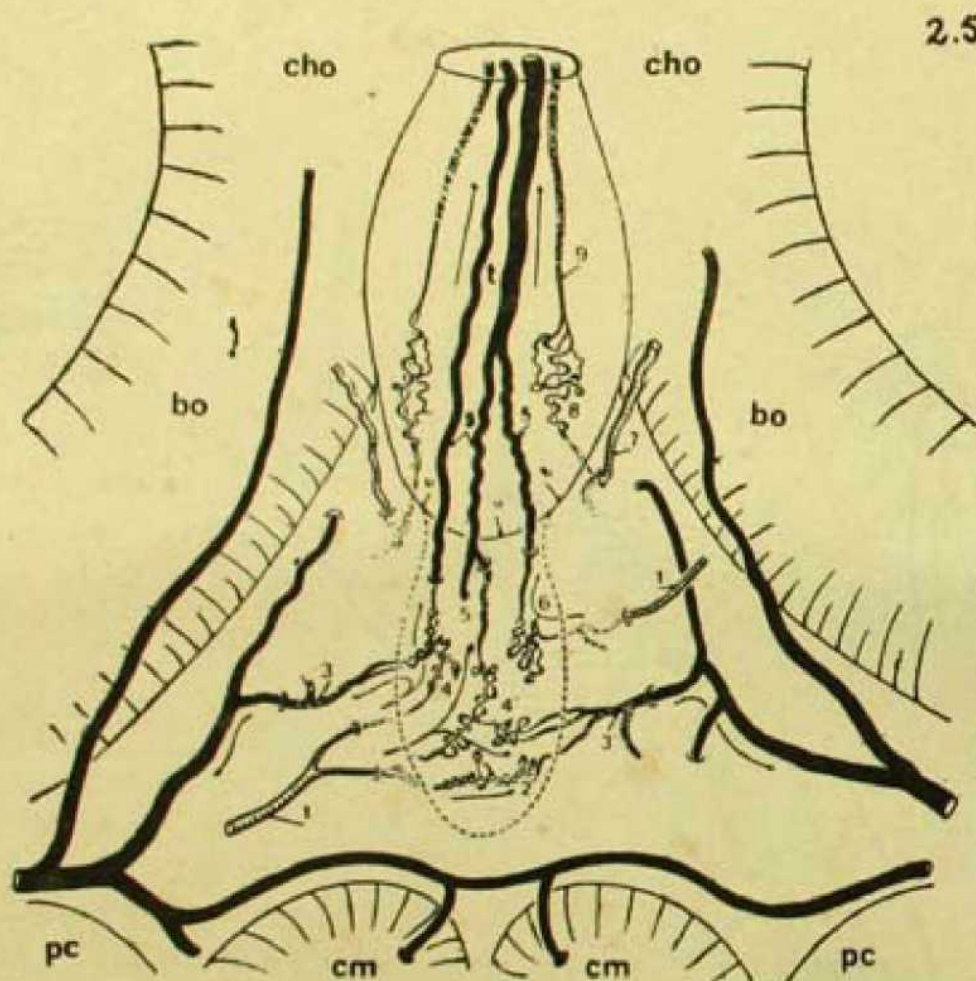


Fig. 2.5. Vascularization of the floor of the diencephalon in man. cho = Optic chiasm, bo = optic tracts, pc = section of the mesencephalon, cm = mammillary bodies, t = hypophyseal stalk, si = sulcus infundibularis. A dotted line surrounds the place occupied by the postinfundibular eminence (PIE). Only the deep network is shown in this drawing. 1 and 1' = Arterioles which supply the PIE. 2 = Deep network exclusively drained by tuberal veins (3'). 4 and 4' = Deep capillary network with mixed drainage via lateral tuberal veins and via long posterior portal vessels (5). 5' = Branch of a portal vessel draining the surface network; this network is not shown in this drawing. 6 = Deep network exclusively drained towards the hypophysis by portal vessels (5). 7 = Branch of the superior hypophyseal arteries which bend over and reach the deep network of the median eminence (8); This network is drained by deep portal vessels (9). —From Duvernoy (1972)

the anterior pituitary. Furthermore, they hypothesized that in much higher concentration the pituitary hormones can reach the brain after coursing through the stalk in a reverse direction. The concentration is never so high through



the general circulation. As there is no blood-brain barrier in the median eminence, peptide hormones diffuse into the brain and CSF from blood. In the rat ACTH and  $\alpha$ -MSH affect memory. Retrograde blood flow through the stalk shunts significant amount of ACTH and  $\alpha$ -MSH to the brain through the median eminence. The authors further speculate that "the vasomotor instability commonly seen in humans at *menopause* may be a consequence of prolonged excessive secretion of gonadotrophins which are delivered to the brain in large quantity by blood flowing retrograde in the pituitary stalk. Other possibilities are also evident."

The blood supply of the human pituitary (Fig. 2.6) has been described in details by Doniach (1977), as reviewed from the description made by Daniel

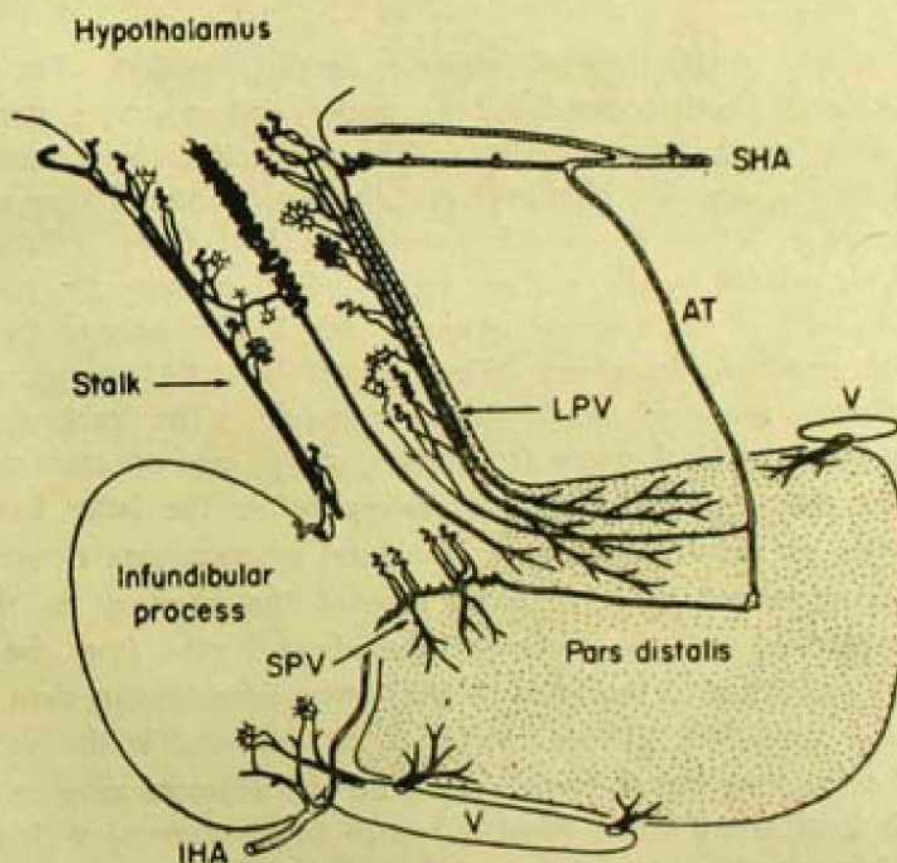


Fig. 2.6. Diagram representing sagittal section of human pituitary, to show main features of blood supply to the various parts. The sinusoids of pars distalis (anterior pituitary) are supplied by two groups of portal vessels, long portal vessels (LPV) draining characteristic capillary loops in the upper infundibular stem (neural tissue of the stalk) and short portal vessels (SPV) draining similar capillary loops in the lower infundibular stem. SHA = superior hypophyseal artery, AT = artery of the trabecula, IHA = inferior hypophyseal artery, V = venous sinus. From Daniel and Prichard (1975) with kind permission of the authors and the editor of *Acta Endocrinologica*.

Courtesy of Professor I. Doniach and W. B. Saunders Co.

and Prichard (1975). The anterior lobe is supplied by the superior hypophyseal arteries, while the posterior lobe is supplied by the inferior hypophyseal arteries.



Both are paired arteries, branches of the internal carotids. The superior hypophyseal artery, one on each side, arises from the internal carotid just after the latter has pierced the dura mater on entering the skull. The two run to the upper part of the pituitary stalk, around which, in the subarachnoid space, they form a ring of ascending and descending branches. These branches pierce the substance of the stalk, where they end in elongated coiled capillary loops. Nerve fibrils of the tubero-infundibular tract end on the walls of these capillaries, pouring down into them the hypothalamic hormones that stimulate and inhibit the anterior pituitary. This primary capillary bed drains into long straight portal veins, which run down along the stalk to empty themselves into the sinusoids of the anterior pituitary. This is how the anterior pituitary receives the hypothalamic hormones, and these chiefly reach the anterior and lateral regions of the anterior lobe.

The blood supply of the trabecula deserves special mention. The trabecula is a connective tissue band, located in the upper and posterior part of the anterior lobe. It is continuous centrally with a connective tissue mass that is interposed between the anterior pituitary, and the lower infundibular stem, and takes the shape of a 'V' in the mid-horizontal section of the pituitary. The hollow of the V is padded by the median wedge, which contains the major mass of the basophilic cells of the anterior pituitary, which also occupy the anterior and anterolateral parts of the gland. The acidophilic cells are grouped into the two posterolateral wings of the V-shaped trabecula. The trabecula receives its blood supply from the loral artery (trabecular artery) one on each side. This artery arises from the superior hypophyseal artery before the latter has reached the pituitary stalk, as described above. The loral arteries run downwards to penetrate the upper surface of the anterior lobe of the pituitary, in the neighbourhood of its junction with the posterior lobe, about 2 mm. from the pituitary stalk. The loral arteries give branches to the lower infundibular stem, wherein they end in coiled capillaries. These capillaries, in turn, end in the short portal veins, which, like the long portal veins, drain into the adjacent anterior pituitary sinusoids. Each loral artery sends down a branch to anastomose with the twigs of the inferior hypophyseal arteries.

The inferior hypophyseal arteries, one on each side, arise from the intracavernous segment of the internal carotids. They run medially to reach the infero-lateral part of the pituitary gland, where they anastomose with each other. This anastomosis takes the form of a vascular ring, located in the groove between the anterior and the posterior lobes of the gland, and provides the only arterial supply to the posterior lobe. It also sends twigs to the lower infundibular stem, one of which anastomoses with a branch of the loral artery, as described above.

The dural capsule of the pituitary, possibly with a little subcapsular glandular substance, is fed by arterial twigs from the hypophyseal arteries as well as from the internal carotids direct.





The venous drainage of both the lobes of the pituitary is into the peripheral venules, which gather into venous sinuses around the gland. These finally drain into the cavernous sinuses of the two sides.



## CHAPTER 3

### DEVELOPMENT OF THE HYPOPHYSIS

The development of the human pituitary gland has been dealt with by Professor Tuchmann-Duplessis *et al.* (1974). It consists of two parts of different origins. The glandular portion is developed from an evagination of the epithelium which covers the vault of the stomodaeum. The neural part is developed from the evagination of the floor of the third ventricle. The induction of the glandular portion is first done by the anterior end of the notochordal system and may be by the prechordal plate. Next the neural primordium or infundibulum is induced by this system. Subsequently the development of each of the two primordia is influenced by reciprocal inductions. That the anterior end of the notochordal system helps as an inductor in development of the pituitary is seen in malformations of the pituitary gland of the type of *doubling*, where there are two neural lobes and two anterior lobes along with doubling of the anterior end of the notochordal system. This has been observed in amphibians, birds and in mammals after experimental manipulations (riboflavin deficiency, hypervitaminosis A, and tranquilizers). The pouch of Rathke is situated in front of the neural primordium. It is attached to the stomodeal vault by pharyngohypophysial stalk which subsequently disappears. Persistence of any of its portion may lead to pharyngeal hypophysis. The entodermal epithelium behind the pharyngeal membrane gives rise to the *pouch of Seessel* (fig. 3.1). In lower vertebrates this may help

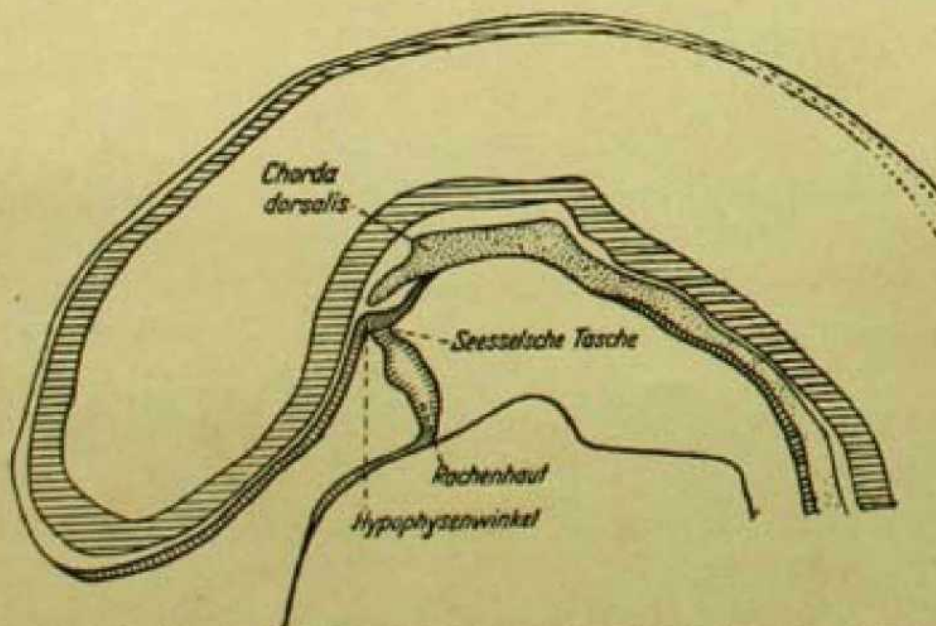


Fig. 3.1. Reconstruction of median sagittal section through a human embryo, 2.5 mm long. Enlargement 1:100 (After Rudel 1918—From Romeis (1940). Courtesy of Springer-Verlag.

in the formation of glandular hypophysis. In primates and man it completely disappears. The anterior wall of Rathke's pouch forms the anterior lobe of the



hypophysis (fig. 3.2). By cellular proliferation this area takes up the shape of a basin which is open above and a median cellular septum divides the basin into

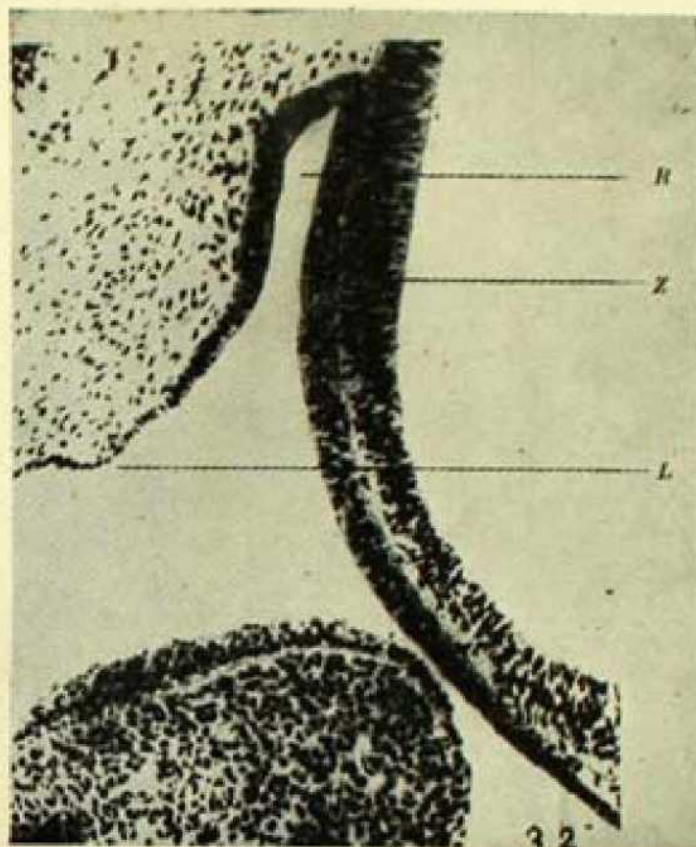


Fig. 3.2. Median sagittal section through the hypophysial region of human embryo, 6.5 mm. long (M.E. 46). (R), Rathke's pouch. X 1:117. —From Romeis (1940). Courtesy of Springer-Verlag.

two compartments. Each one is known as fossa of Atwell. They are filled with mesenchyme. Peripheral growth leads to obliteration of the fossae. The median septum forms the medial part and the lateral portions form the pars lateralis of the anterior lobe. With the proliferation of the median septum upwards and the cellular expansion surrounding it, the pars tuberalis is developed. The development of the posterior wall is very little in man. The pars intermedia is formed from it and the cells enter into the anterior lobe and this lobe has disappeared more or less by puberty. This cavity of Rathke's pouch forms the pituitary fissure.

Evagination from the floor of the third ventricle forms the infundibulum. The neural lobe is attached to the diencephalon by a thin hypophysial stalk.

Wingstrand(1951, 1966) described the development of the pituitary gland (fig. 3.3). The pouch of Rathke which is the primordium of the anterior pituitary is hollow and the top is in contact with the developing neural lobe. The pouch becomes broad near the top and near the base and thus forms an aboral and an oral lobe(1951). They are separated by a constriction. In the adult an epithelial stalk marks the connection with the oral ectoderm. A pair of processes (lateral lobes) grow out from the oral lobe near the constriction and bend up-



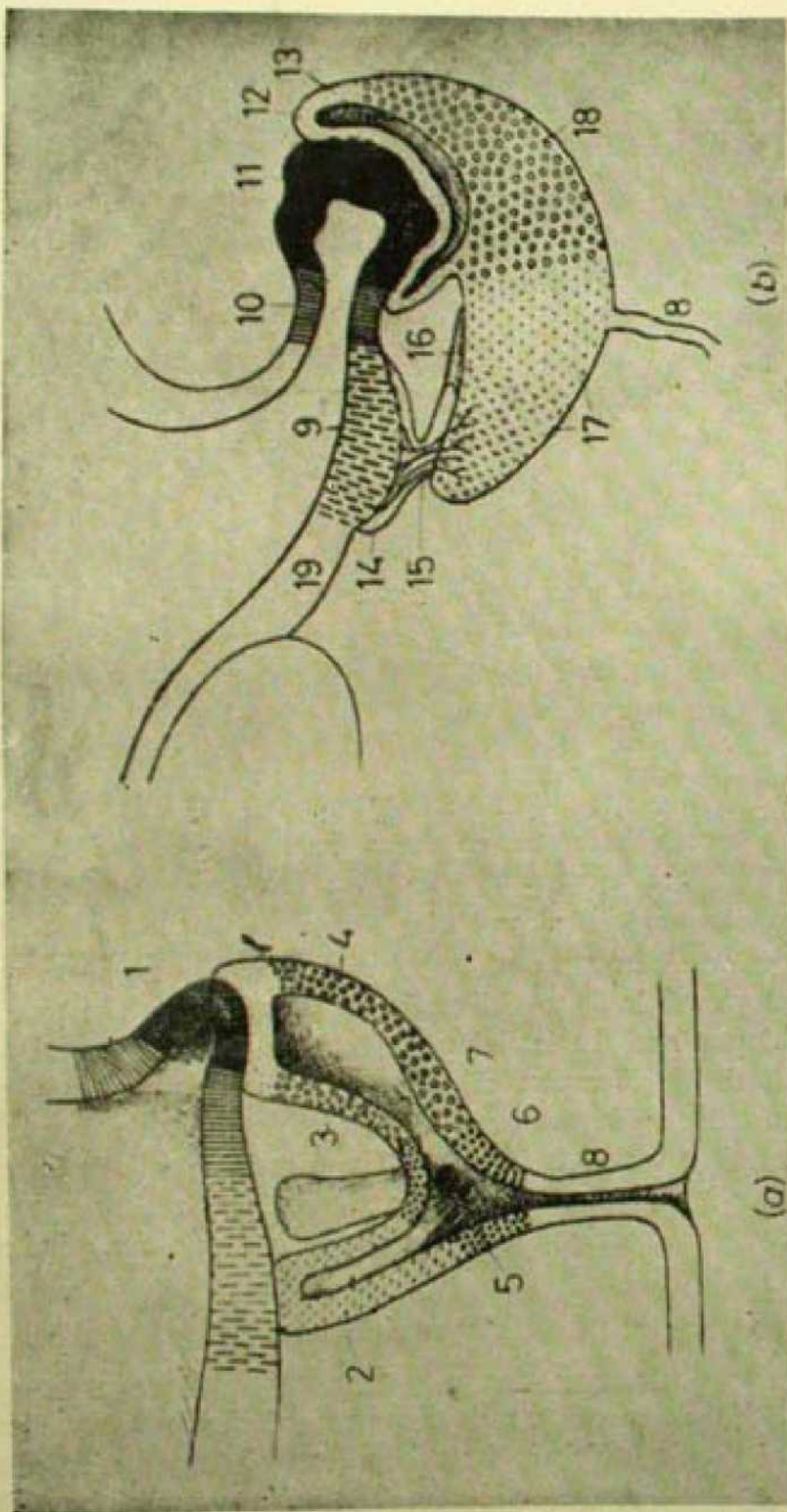


Fig. 3.3. Diagrams showing the structure of a generalized amniote pituitary (b) and its embryonic origin (a) as seen particularly, in reptiles. (1) saccus infundibuli; (2), anterior process; (3), lateral lobe; (4), aboral lobe; (5), opening of lateral lobe cavity; (6), oral lobe; (7), constriction of Rathke's pouch; (8), epithelial stalk; (9), median eminence; (10), infundibular stem; (11), neural lobe; (12), pars intermedia; (13), hypophyseal plate; (14), juxtaneural pars tuberalis; (15), portotuberal tract; (16), pars tuberalis interna; (17) cephalic lobe of pars distalis; (18), caudal lobe of pars distalis; (19), pars oralis tubercis. Courtesy of Professor K. G. Wingstrand and Butterworths.



wards towards the median eminence. An anterior process arises from the oral lobe in many forms. The process may be so large that the adenohypophysial anlage takes up the shape of a U in a median section. The anterior process is in contact with the future median eminence but later on the contact may be disrupted.

The part of Rathke's pouch having contact with the neural lobe gives rise to the pars intermedia but there is no pars intermedia in birds and a few other species. The oral lobe and the part of the aboral lobe which does not take part in the formation of the pars intermedia, together form the pars distalis. The lateral lobes get attachment with the median eminence and form adult pars tuberalis. In birds the pars distalis may get attachment caudally with the neural lobe, where there is no pars intermedia. A zona tuberalis in mammals extends into the gland having a continuation with the pars tuberalis. It does not correspond to any embryonic anlage and the predominant cell type is basophilic. This histological differentiation is due to the humoral influence of the portal blood having its entry into the pars distalis through the zona tuberalis. This zone is also noted around the entrance of portal vessels in some reptiles and amphibians.

The postoptic part of the hypothalamic floor gives rise to the neurohypophysis. The saccus infundibuli is evaginated from the posteroventral hypothalamic wall below the tuberculum posterius and the recessus posterior. In amniotes, proliferation from the end of the saccus infundibuli leads to the formation of the adult neural lobe. The base of the saccus infundibuli forms the infundibular stem. "The median eminence is formed by the hypothalamic wall in front of or around the base of the saccus. The neural lobe is in close contact with the adenohypophysis at least in early embryonic stages (distale adenoneurohypophysare Kontaktfläche of Spatz, Diepen and Gaupp, 1948; Spatz, 1954)". The saccus infundibuli becomes forked or T-shaped at the end and two primary branches are formed thereby. By thickening of the walls of these primary branches the neural lobe may grow, or it may be formed by *diffuse migration of the nervous material into the surrounding mesenchyma*. Thus a solid lobulated neural lobe is formed in mammals and snakes. In these cases the lumen of the recessus infundibuli (neural lobe) may get smaller or it obliterates. There are vessels and connective tissues. The ependymal cells leave their layer and become free pituicytes. In *Sphenodon*, many reptiles and birds the neural lobe is wrinkled and thin walled. It is hollow and lobulated. At the ventricular surface there is an ependymal layer, an intermediate fiber layer comprising preoptico-hypophysial tract and the palisade layer is superficial. No free glial cells are found here. In between the two extreme types of neural lobe i.e. compact and hollow, there are many intermediate types. At the median eminence there is a *proximale neuro-adenohypophysare kontaktfläche* of Spatz and his school.



## CHAPTER 4

### MAMMALIAN HYPOTHALAMUS, MEDIAN EMINENCE AND THE INFUNDIBULAR PROCESS

Diepen(1962), Christ(1966), Sloper(1966), Holmes and Ball(1974) and others reviewed this subject. In the hypothalamus there are several nuclear groups. In mammals several cell groups form the supraoptic nucleus and they are joined together by strands of scattered cells. The larger portion of the nucleus is called the pars dorsolateralis and it is situated in front of the optic chiasma. The pars ventromedialis is the smaller, compact postchiasmatic part. It is situated at the posterior surface of the optic chiasma. Accessory supra-optic nuclear groups connect the nucleus supraopticus and the nucleus paraventricularis. The retrochiasmatic division belongs to a different system(Szentagothai, Flerko, Mess and Halasz, 1962) because (1) their axons do not join the supraopticohypophysial tract, (2) these cells in the rat were beautifully stained with their whole dendritic arborizations by Golgi and Cox methods but the true neuro-secretory magnocellular nuclei could not be successfully impregnated by these methods, (3) the intercellular meshwork of preterminal collaterals is very scanty in the supraoptic and paraventricular nuclei but very rich in this area having characteristic synapses, (4) NSM was absent in this nuclear group whereas it is present in the supraoptic nuclear group, and (5) after injection of ( $^{35}\text{S}$ ) -methionine, the anterior part of the nucleus showed strong activity in the radioautographs, but there was no activity in the retrochiasmatic portion.

Variation in the activities of the prechiasmatic and postchiasmatic divisions of the supraoptic nucleus is noted after stalk section, dehydration experiments and destruction of the neurohypophysis. These variations are only quantitative in nature and they can be explained by different lengths of the respective nerve fibres (Christ, 1966; Diepen, 1962).

Unmyelinated nerve fibres proceed through the neural stalk into the posterior lobe (reviewed by Christ, 1966).

Greving(1926) described a fibre tract arising from the paraventricular nucleus called the *tractus paraventricularis cinereus*, in addition to the supraoptico-hypophysial tract. Diepen(1962) considered the common phylogenetic origin of the supraoptic and paraventricular nuclei. The fibres from the paraventricular nucleus proceed toward the supraoptic nucleus in a curved way and more or less of these fibres end in the supraoptic nucleus. This is proved by the fact



that after stalk section, there is partial loss of cells in the paraventricular nucleus. Rasmussen(1940) concluded that the paraventricular nucleus sends only a few fibres to the infundibular process and most of the fibres end in the supraoptic nucleus and the median eminence. Palay(1953) and Diepen and Engelhardt(1958) thought that the paraventriculohypophysial tract is formed by fibres of different lengths and they terminate at different levels in the hypothalamus and neurohypophysis. So, lesion in the distal part of the neural stalk will lead to a damage of only small number of long paraventricular fibres; but the lesion of the median eminence will damage both the long and short fibres.

In the rat, cat, dog etc. "the rostral tip of the paraventricular nucleus, which consists of comparatively small number of cells, is situated in the rostral hypothalamic region close to the third ventricle and above the level of the supraoptic nucleus. From there it extends in a dorsocaudal direction, with its posterior pole reaching as far dorsal as the dorsal hypothalamic area" Christ(1966). In man the position of the long axis of the paraventricular nucleus is more vertical. The fibres arise from different parts of this nucleus and proceed toward the median eminence by different circuitous routes and thus they do not form a single fibre tract.

Works of Scharrer(1928) and Bargmann(1949 and subsequent papers) clearly demonstrated the secretory phenomena in the hypothalamohypophysial system (Gomori's chrome-alum-haematoxylin method) (fig. 4:1). The hypothalamohypophysial system comprises of supraopticohypophysial tract and the paraventriculohypophysial tract. These together form the neurosecretory system. The secretory neurons produce vasopressin and oxytocin. Olivecrona(1957) thought that oxytocin is produced in the paraventricular nucleus. Other staining methods included aldehyde fuchsin. Deep staining of the neurosecretory material takes place. It also stains lipofuscins. Sloper(1955) used a modified thioglycollate-ferric ferricyanide method (Adams, 1956). Adams and Sloper(1956) used the method which was due to oxidation of cysteine to cysteic acid when acted upon by performic acid and this was followed by staining with alcian blue at a low pH. Control sections comprised unoxidised ones and they are to be stained in a similar way. Autoradiographic studies have been also performed because the neurohypophysis contains sulphur-rich material (Arnott and Sloper, 1958; Sloper, Arnott and King, 1960); Ficq and Flament-Durand, 1963).

### *The tuberohypophysial tract*

The supraopticohypophysial system or the posterior lobe system has a *neurovascular chain* (Harris, 1944, 1948, 1955, 1961) and Green and Harris(1947). The tuberohypophysial system consists of the nerve tracts, capillary loops of the median eminence, neural stalk and hypophysiportal vessels (Christ, 1966). This system may be identical with the *neurovascular chain system*.



Roussy and Mosinger(1946) in *Traite de Neuro-Endocrinologie* said that the following parvocellular nuclei and areas are sources for the tuberohypophysial tracts. They are : inferior periventricular nucleus of the anterior hypothalamus or nucleus of the infundibulum (infundibulohypophysial fibres), nucleus infero-internal and nucleus supero-internal of the anterior hypothalamus and in man the main nucleus of the tuber (tuberohypophysial fibres), anterior hypothalamic segment of the hypothalamomammillary nucleus, ovoid nucleus, internal nucleus of the preoptic zone (preopticohypophysial fibres). Spatz(1951, 1958), Nowakowski(1951), Christ(1951), Wingstrand(1951), Diepen(1953), Oksche(1960, 1961), and Szentagothai *et al.*(1962) thought that the nucleus infundibularis is the main source of the tuberohypophysial tract. Martinez(1960) thought the nucleus infundibularis, nucleus ventromedialis, nucleus paraventricularis, nucleus hypothalamicus ventrolateralis, nucleus hypothalamicus posterior, and lateral hypotha-



Fig. 4.1. Nucleus supraopticus of the dog (CAHP stain)  $\times 550$ . Shows neurosecretory cells in different stages (Bargmann, 1954). Courtesy of Professor R. Diepen and Springer-Verlag.

lamic area contribute to the fibresystem of the tuberohypophysial tract. According to Dellmann(1962) the contributing areas are : nucleus infundibularis, nucleus ventromedialis, nucleus dorsomedialis, posterodorsal periventricular hypothalamic area, nucleus lateralis tuberis, lateral area of the posterior hypothalamus, ventro-



lateral hypothalamic area, hypothalamic premamillary area, and the tuberomammillary nucleus. Christ(1951) found delicate nerve fibres to take origin from the cells of the ventromedial and arcuate nuclei and to proceed toward the median eminence.

Sagittal section through the median eminence and the neural stalk of the cat stained with Bodian's method (Nowakowski, 1951) demonstrates the un-myelinated nerve fibres and the infundibular structure (fig. 4:2). The ventral wall of the infundibulum has two layers (fibre layer and glandular layer). In the inner layer there are coarse nerve fibres and very delicate fibres. These delicate fibres may be collaterals of the supraopticohypophysial tract or preterminal and terminal fibres of the tuberohypophysial tract.

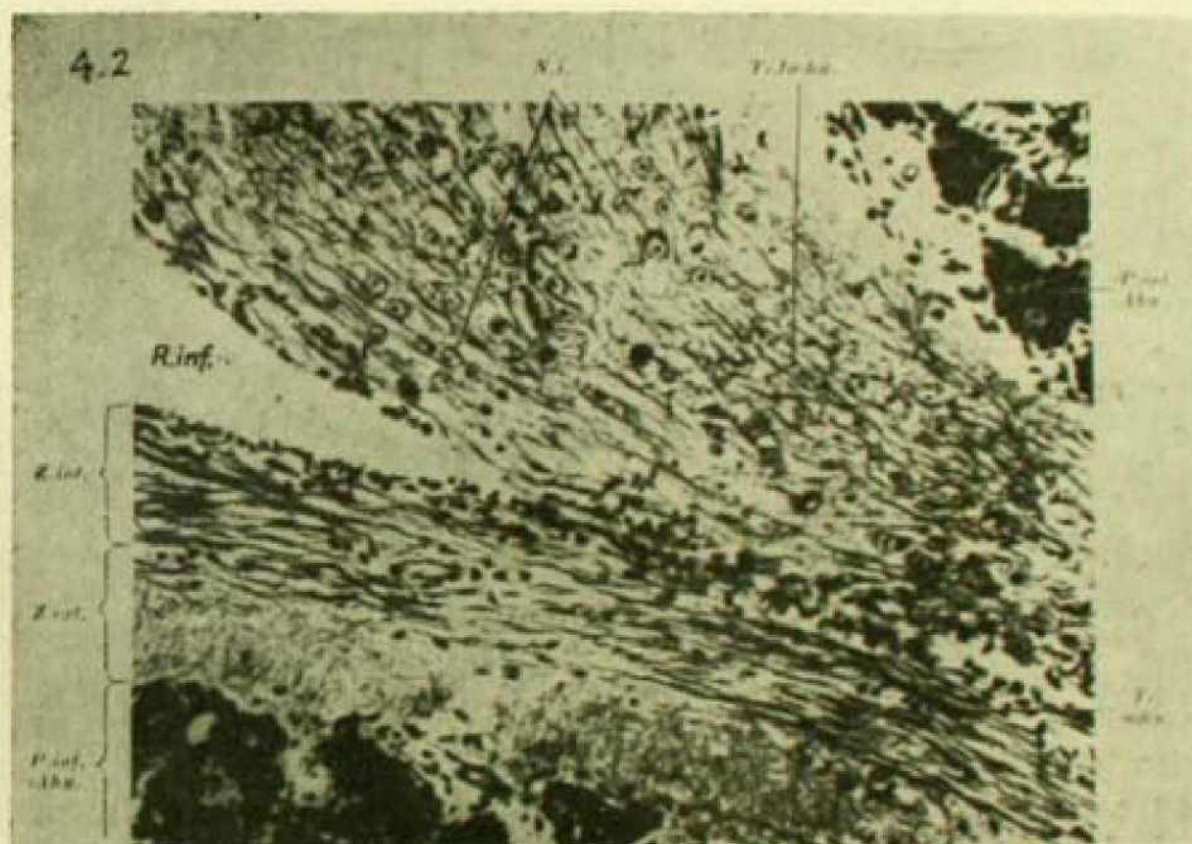


Fig. 4.2. Infundibular zone of the cat. Bodian preparation (sagittal section  $\times 170$ ). (Z. int.), Zona interna infundibuli; (Z. ext.), Zona externa infundibuli. There is endplexus of Tr. tubero-hypophysius; (Tr. tu. hy.), Tr. tubero-hypophysius; (Tr. sohy.), Tr. supraopticohypophysius (thick fibres); (N.i.) Nucleus infundibularis; (R. inf.) recessus infundibularis; (P. inf. Ahy.) Pars infundibularis of the adenohypophysis. Courtesy of Professor R. Diepen and Springer-Verlag.

Human neural stalk and infundibular process contain *Greving's islands* which are perivascular spaces formed by the arching of the fibre bundles of the hypothalamohypophysial nerve fibres. Different types of pituicytes and epithelial elements are also noted.

Diepen(1962) studied the difference between the neurosecretory pictures in various mammals. In silver preparations varying degrees of changes in the



neurosecretory fibres have been noted (fig. 4.3). The figure shows different degrees of local swellings. Smaller swellings may be reversible. There is inter-

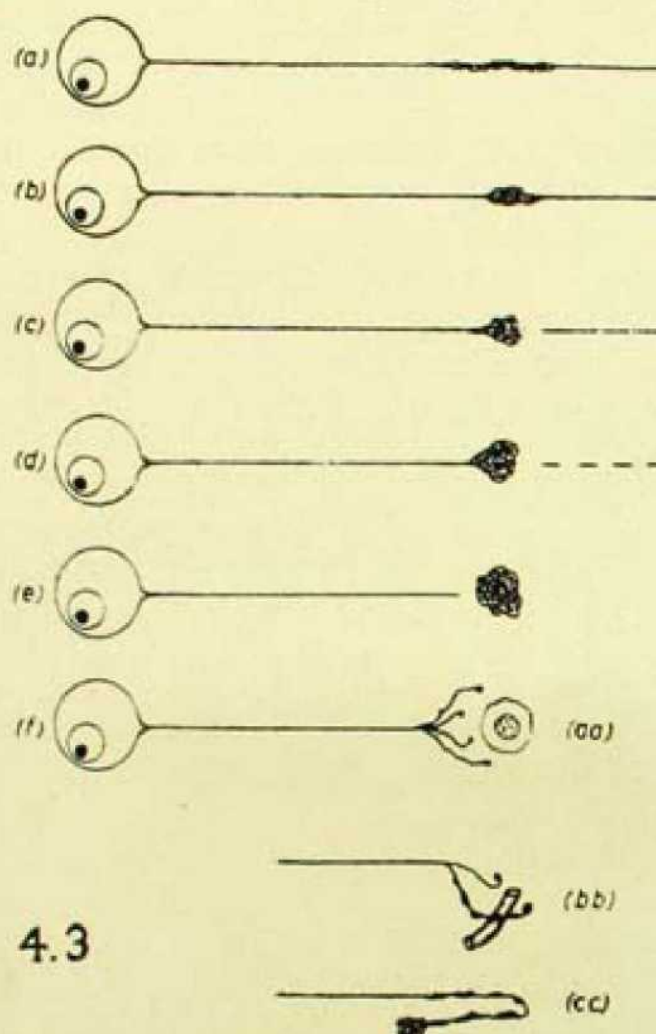


Fig 4.3. Schematic representation of different degrees of local swellings in neurosecretory nerve fibres. Courtesy of Professor R. Diepen and Springer-Verlag.

ruption (c) in the axonal continuity when the local swelling is large. If it happens then the distal portion of the axon undergoes Wallerian degeneration(d). There may be interruption between the continuity of the proximal portion of the axon and the swelling (e) and the local swelling becomes an isolated bulbous or ball-shaped fragment (f, aa). Neurofibrils may grow out from the proximal stump. These regenerating fibres may show beading and grow irregularly toward adjacent blood vessels (bb). The Gomoripositive fibres in the external zone of the median eminence may be such stray regenerating fibres. Gomoripositive Herring bodies may correspond to local alterations in silver preparation. Diepen thinks that neurosecretion is also due to a local process in the axon. The disintegrating phenomena are more prominent in the posterior lobe of the dog. Diepen's concept speaks about a local production of nsm but it does not deny the existence of a proximo-distal flow of axoplasm (Weiss and others) (Holmes, 1961, 1963).

Christ(1962) noted that after complete interruption of the hypophysial stalk in the rabbit, there was accumulation of neurosecretory material (nsm) not only



in the proximal stump but also in the distal stump which speaks in favour of a local secretory activity of the respective parts of the neurons.

Regeneration phenomena happen after circumscribed lesion of the tractus supraoptico-hypophysius in the infundibulum of the rat (Diepen, 1962). There were regenerating fibres in the proximal stump which reached the infundibular special vessels in the outer zone of the infundibulum. They reach the highly vascular pars infundibularis adenohypophyse (fig. 4.4).

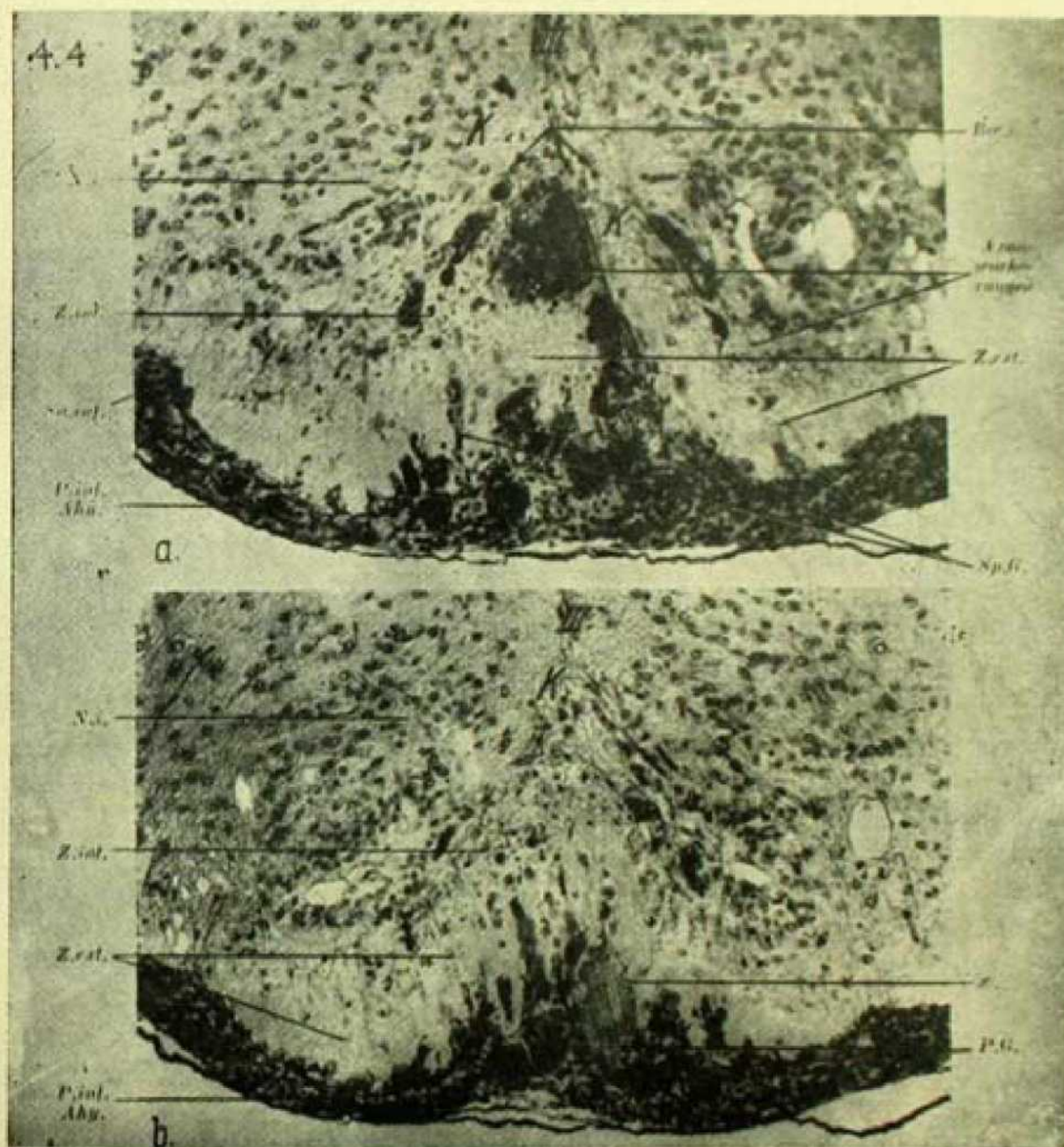


Fig. 4.4. Proximal hypophysis of a rat 25 days after coagulation (K.) in nucleus a & b. infundibularis (Ni.). (Z.int.) inner zone of the infundibulum. CAHP stain after Gomori. X 150.

(a) Site of lesion with neurosecretion containing beaded fibres and Herring bodies (Tr. supraoptico-hypophysius: Axonal exuberance).

(b) Section through the proximal lesion site. Exuberance of axonal regeneration in the outer zone (Z.ext.) of the infundibulum which is in contact with the Pars infundibularis of adenohypophysis. (Rec. i.) Recessus infundibuli. (Su. inf.) Sulcus tubero-infundibularis. (Sp. G.) Special blood vessels. (P.G.) Portal vessels (After Engelhardt and Diepen, 1958). Courtesy of Professor R. Diepen and Springer-Verlag.



Diepen(1962) discussed the structure of the proximal hypophysis (infundibulum and pars infundibularis adenohypophyse). The internal infundibular zone contains the tractus supraopticohypophyseus. It is rich in pituicytes. The external infundibular zone has contact with the pars infundibularis adenohypophyse and the proximal adenoneurohypophyseal contact area is formed. There are special blood vessels (capillary loops) and the mantelplexus. The tuberohypophyseal fibres in the external infundibular zone are Gomorinegative and the granules are argyrophilic. There are also ependymal fibres and subependymal glial fibres.

Diepen(1962) described the relationship between Gomori-preparation and silver-preparation of the supraopticohypophyseal system in dogs.

Regarding the release of posterior lobe hormones and the role of pituicytes Diepen(1962) gave a schematic drawing in his book in fig. 274.

#### *Vasopressin and oxytocin in hypothalamic nuclei*

Abel(1924) first reported that the mammalian hypothalamus contains vasopressin and oxytocin-like substances. This was subsequently confirmed by van Dyke(1926), Trendelenburg(1928) and Vogt(1953). Bargmann and Scharrer(1951) reported that the hormones are produced in the nerve cells of the supraoptic and paraventricular nuclei of the mammalian hypothalamus or the preoptic nucleus of lower vertebrates. They are carried by axoplasmic flow in the nerve fibres and stored in the pars nervosa. Green and Maxwell (1959) thought that the modification in the mitochondria all along the axon produced the hormones. They conducted ultrastructural studies and they suggested that oxytocin is stored in neurosecretory vesicles lacking electrondense centres.

Lederis(1962) used a paper chromatographic procedure and showed that vasopressin and oxytocin are present in the mammalian hypothalamus. In the hypothalamus the hormone content is 1/50th of that of the neural lobe. The vasopressin : oxytocin (v/o) ratios in the hypothalami of man, ox, pig, rabbit and rat did not differ much from those found in the neural lobes of the same animals. In the dog v/o ratio in the hypothalamus was very high compared to that in the neural lobe. In an Indian elephant similar feature was observed. Dog's hypothalamus contained oxytocin. In the supraoptic nuclei more vasopressin than oxytocin was found and more oxytocin was found in the paraventricular nuclei. Vasopressin is synthesized in the supraoptic nuclei predominantly or entirely and synthesis of oxytocin occurs in the paraventricular nuclei. In the dog and in the Indian elephant, synthesis of oxytocin occurs throughout the length of the neuron. In rabbits kept on a dry diet for 14 days there was a considerable fall in the hormone content of the neural lobe. There was depletion of vasopressin more than that of oxytocin. The v/o ratio and the absolute hormone content in the hypothalamus of dehydrated rabbits were not greatly different from those in normal controls.



*Electron microscopic studies*

Ultrastructural observations of Gerschenfeld, Tramezzani and De Robertis(1960) and Palay(1960) in the neurohypophysis of mammals, birds, reptiles and amphibians revealed nsm along the axons and at the terminals to be composed of dense granules of about  $0.1 \mu$  in diameter surrounded by a membrane. De Robertis(1962) studied the neurosecretory axons in four different regions of the toad : (I) in the hypothalamus, (II) in the hilar areas of the infundibular process, (III) near the capillaries and (IV) at the ending proper (fig. 4.5).

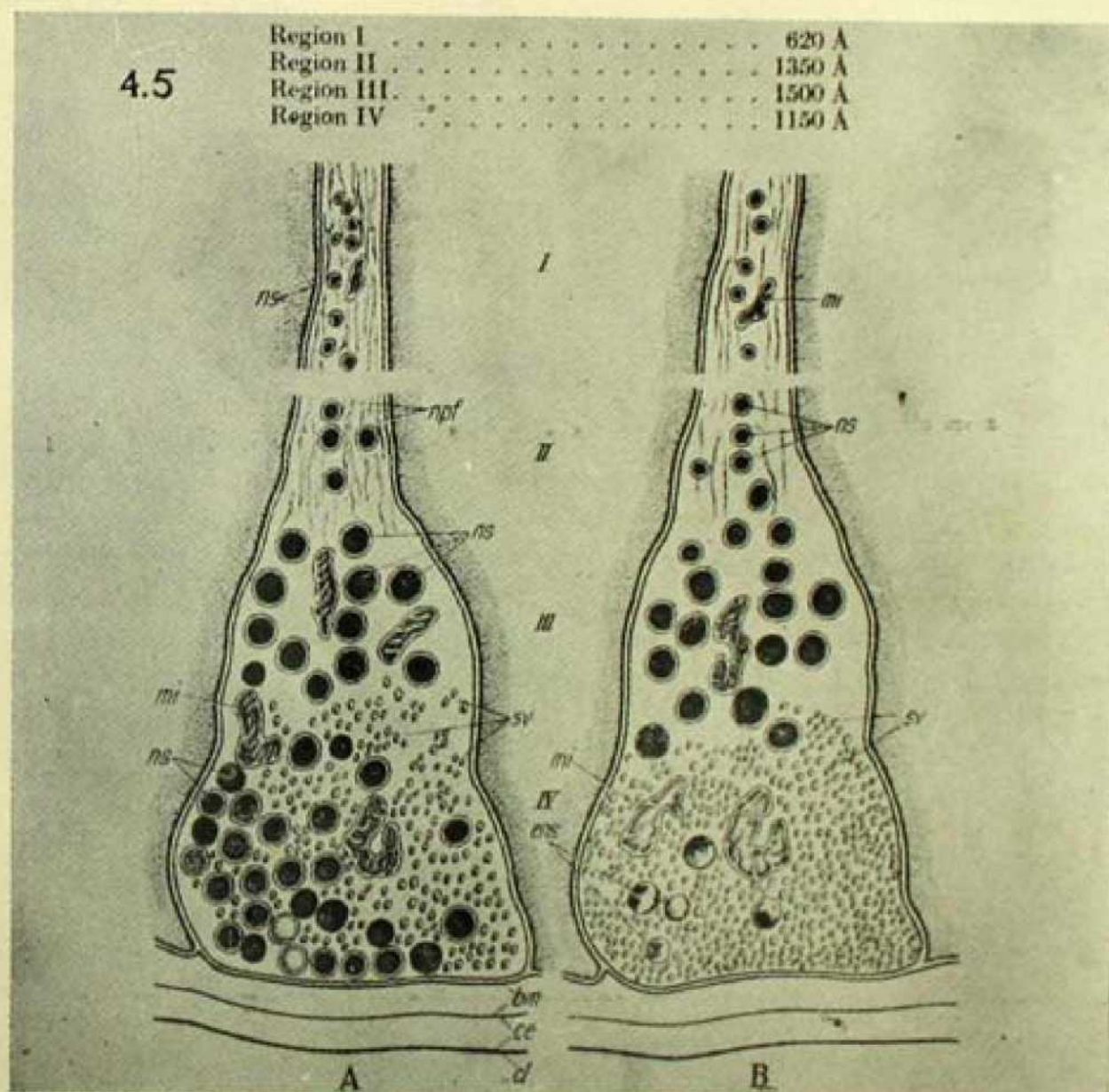


Fig. 4.5 Diagram of the different regions (I, II, III & IV) of a neurosecretory axon. (A), control animal. (B), in a chronically dehydrated toad. To note the release of nsm from region IV and the increase in the number of synaptic vesicles (SV); (npf), neuroprotofibrils; (mi), mitochondria; The relative size of the granules in the different regions, and of the synaptic vesicles is maintained. (bm), basement membrane; (ce), capillary endothelium; (cl), capillary lumen (Gerschenfeld, *et al.*, (1960). Courtesy of Professor R. Diepen and Springer-Verlag.



In region I, there was active formation of secretory granules within dense vesicles. The secretory units grow from a mean size of 620 Å in region I to 1350–1500 Å in regions II and III reaching a rather uniform (quantal) size. Increase in the volume of the granules takes place from region I to II and III.

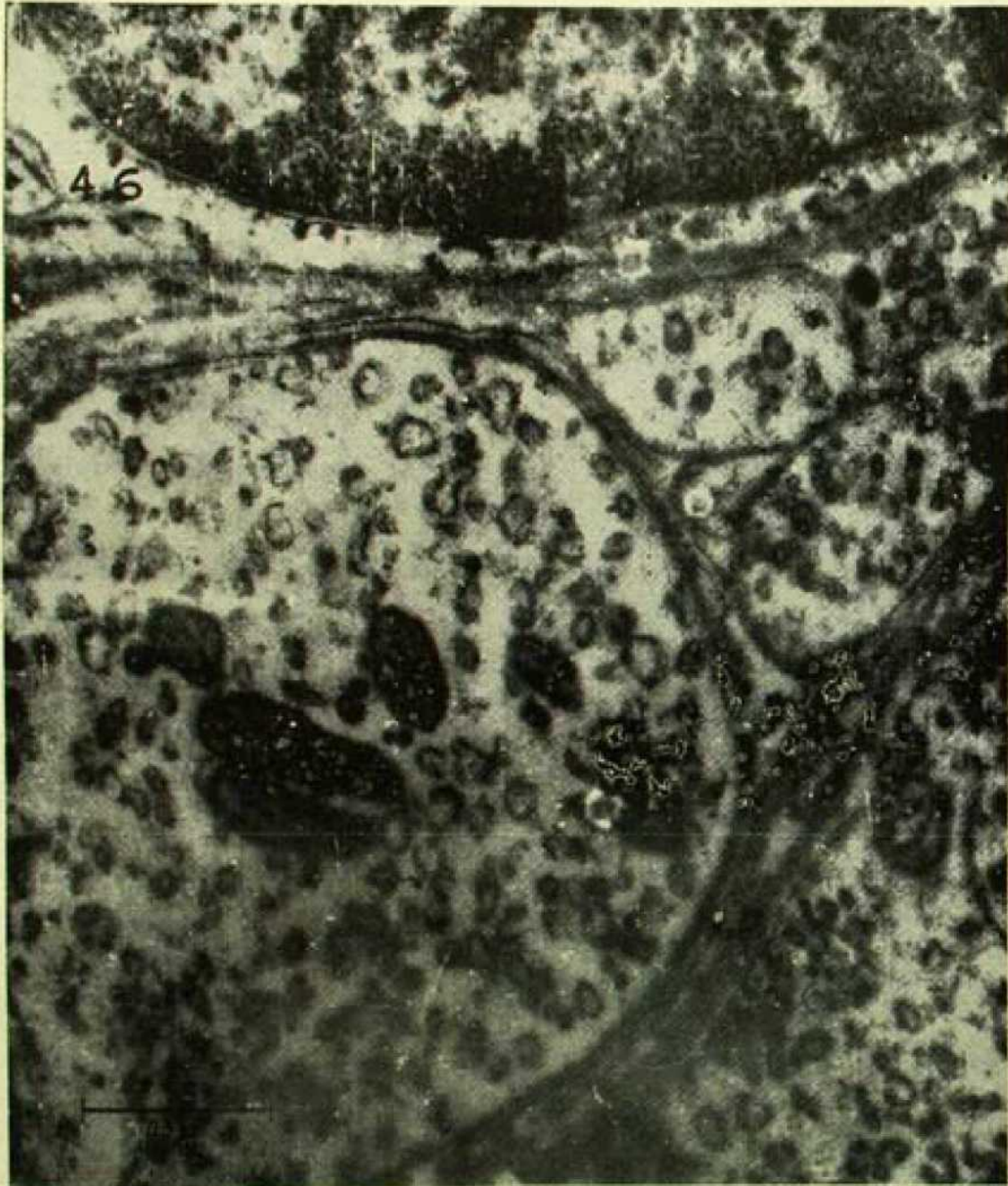


Fig. 4.6. Nerve fibre in the posterior lobe of a rat 30 minutes after Histamine injection. The neurosecretory granules look optically empty. The synaptic vesicles are unchanged. (Ultrastructural view.  $\times 52000$  times) (After Hartman, 1958). Courtesy of Professor R. Diepen and Springer-Verlag.

At the endings (region IV) a component appears which is morphologically similar to the synaptic vesicles with a mean diameter of 420 Å. In the axons of regions I to III there are plenty of neuroprotofibrils and thinner neurofilaments and some mitochondria. The mitochondria had no relationship to the nsm as stated by Green and Maxwell (1959).



The neurosecretory granules in chronically dehydrated animals disappear from the endings but are present in other regions of the axon. The synaptic vesicles persist at the endings and increase in number. With massive discharge of nsm in acutely dehydrated animals or during ether anaesthesia, there is diminution in the number of synaptic vesicles. With the release of neurosecretion the granules do not disappear. There are less dense granules or empty membranes for some time in the endings (fig. 4.6).

De Robertis(1962) found also a progressive synthesis of the material along the axon until a maturation size of the neurosecretory granule is attained. Axoplasmic streaming of course takes place.

The synaptic vesicles in some way elicit the actual release of nsm from the ending as is noted in the nerve endings in the adrenal medulla. Acetylcholine is liberated at the terminals in the neurohypophysis and this releases oxytocin or vasopressin.

Heller(1961) said that the differential release of the two neurohypophysial hormones could be explained by assuming the presence of oxytocinergic and vasopressinergic neurons. Subcellular fractionation of the neural lobes of different species indicates that the oxytocic and vasopressor activities are located in different fractions which contain different types of granules or different kinds of nerve endings(Heller and Lederis, 1961; Kauz and Lederis, 1965; Bindler, LaBella and Sanwal, 1967). Two types of neurosecretory nerve fibres were found in the neural lobe(Lederis, 1964; Campbell and Holmes, 1966; Zambrano and De Robertis, 1968; Rodriguez and La Pointe, 1969). Sokol and Valtin(1967) found absence of nsm from some areas of the neural lobe of rats having hereditary diabetes insipidus. NSM containing fibres are oxytocinergic. Non-NSM containing fibres are due from the defective neurons which do not synthesize vasopressin.

Rodriguez(1971) found type I and type II fibres (non-neurosecretory) in small numbers in the bovine neural lobe. Two types of neurosecretory fibres could be distinguished. Type III fibres contain granules of 180-220 nm in diameter. A wide space separates the limiting membrane from the dense core. A crystalloid structure can be seen in the dense core of several of these granules. The limiting membrane of some granules is connected to short tubules like a tail. The type IV axons have very pale granules of 180-220 nm in diameter. The outline is more regular than that of granules in Type III fibres. Amidst these granules there are small dense bodies (about 150 nm in diameter). The halo between the dense core and the membrane is absent in them. These dense bodies are completely different from the dense granules of the Type III axons. Type IV fibres were more than Type III fibres. They (Types III and IV) were also present in the bovine infundibulum.





In the rat and bovine neural lobes the ependymal component was completely absent. There were plenty of pituicytes. Pituicyte processes were more than nerve fibres. Pituicyte processes intervene between the nerve endings and blood vessels and they form an incomplete barrier (Rodriguez, 1971).

Mast and plasma cells and fibroblasts are abundant in the rat neural lobe and are numerous (specially mast cells) in the bovine neural lobe. The connective tissue cells are situated in the perivascular spaces.

Rodriguez(1971) thought that the pale and the dense neurosecretory granules are of different types and do not represent different physiological states of the same type of granule. The dense granules contain AVP and the pale granules contain oxytocin in the bovine neurohypophysis. In the monkey similar feature happens.

Douglas, Nagasawa and Schulz(1971) studied the mechanism of secretion of posterior pituitary hormones and the significance of microvesicles (synaptic vesicles). The secretion was by *exocytosis* and the formation of microvesicles was a by-product of the process.

### *Herring body*

Basophilic colloid masses in the neurohypophysis and neural stalk was noted by Herring (1908). They were thought to be derived from basophil cells migrating into the neural lobe from the adenohypophysis. They could proceed towards the hypothalamus through the neural stalk. Thus *Herring bodies* were described but a confusion remained that they were cells. The colloid in the posterior pituitary could be stained by neurosecretory stains with chromealum haematoxylin or aldehyde fuchsin. With these stains it could be found that Herring bodies are accumulations of neurosecretory material either along or within the neurosecretory axons.

Green(1966) observed ultrastructurally pleomorphic character of the neurosecretory material in the Herring body of normal rat pituitary. The material was dense and of large vesicular type. There were mitochondria, ballooned structures, dense structures and numerous vesicles. Disorganized mitochondria and curious lamellated structures were also seen. The lamellation of dense bodies were suggestive of membrane replication.

Kurosumi(1974) said that ultrastructurally the neurosecretory granules are found within the nerve fibres and so the idea of light microscopists that the transport of the neurosecretory substance takes place along the outer surface of the nerve fibres of the hypothalamo-hypophysial tract should be abandoned. Neurosecretory granules filling up axonal swellings ultrastructurally correspond to the Herring bodies. Lysosome-like inclusions have been noted within the axons of the posterior pituitary. They stain dark with basic dyes under light microscope and Kurosumi said that they might be degenerated axons and under light micros-





cope they could be identified as Herring bodies. Secondary lysosomes can be formed by fusion of elementary granules of nsm and they are found in some axons. Therefore, lysosome-like inclusions could develop partly from the neurosecretory granules. Kurosumi thought that this phenomenon could correspond to crinophagy (Farquhar) in adenohypophysial cells.

### *Pituicytes*

Bucy's pituicytes were seen in the neural lobe. In the rat these cells have numerous fat droplets. The osmiophilic granules accumulated in the pituicytes in water-deprived rats. Romeis classified the pituicytes into reticulopituicytes, micropituicytes, fibre pituicytes and adenopituicytes. The adenopituicytes were plump cells and mostly without processes. They were rich in cytoplasm having granules or they had vacuolated appearance. These indicated a secretory activity. The pituicytes are not the source of antidiuretic hormone.

Dellman(1962) described two main types of pituicytes: fibre pituicytes and protoplasmic pituicytes. Cell bodies and processes of protoplasmic pituicytes sometimes have granules of various size in different cells. Romeis(1940) described them as pigmented pituicytes. Christ(1966) demonstrated the variability in size and shape of the pituicytes in the bovine neurohypophysis.

### *Fibre pituicytes*

Cytoplasm is poor in the cell body. Varying amount of chromatin is contained in the oval shaped nuclei. At times clear nucleus with only a few dark granules is noted. Sometimes the nucleus cannot be distinguished separately from the dark stained cytoplasm. Fibre pituicytes have long (107 $\mu$ ) processes.

### *Protoplasmic pituicytes*

Romeis described them as micropituicytes. Though they were thought to be micro, yet considerable variation has been noted in size and shape. The macro types are more common in the posterior pituitary. These polymorphic cells are rich in cytoplasm with several processes. Nuclei are either oval or round. They contain variable amount of chromatin granules.

The processes of fibre pituicytes and protoplasmic pituicytes end either on connective tissue or on blood vessels with end feet.

Christ considers the pituicytes as glial elements but considerable variation in size and shape has been noted in them, unlike the glia cells in other parts of the central nervous system. Intermediate forms between protoplasmic pituicytes and fibre pituicytes can also be seen. Embryonic forms resembling an astroblast can also be noted.



Ultrastructurally only two types of pituicytes could be demonstrated in the posterior pituitary of the rat. They were called protoplasmic pituicytes and fibrous or reticular pituicytes by Kurosumi *et al.* (1964). The processes of reticular pituicytes do not contain any special filamentous substance and they embrace neurosecretory axons like oligodendrocytes in the brain or Schwann cell in the peripheral nerves (Kurosumi, 1971).

Ultrastructurally the protoplasmic pituicyte contains many lipid droplets, mitochondria, RER but the smooth ER is difficult to find out. There are no evidences of hormone production in these cells, neither it is possible that synthesis of the lipid occurs in this cell. The lipid droplet is ingested from outside the pituicytes (Kurosumi, 1974). NSM containing the posterior pituitary hormones is discharged strongly from nerve terminals and axons of the posterior pituitary by water deprivation. After release of nsm there was increased accumulation of lipid in the pituicytes. Similar features occurred after dehydration, delivery and stress (Kurosumi *et al.* 1964). The lipid droplets of pituicytes are not neutral fat. They are mostly phospholipid. Shortly after dehydration, pinocytosis occurs. The coated pinocytotic vesicles have been found by Kurosumi (1974) near the surface of pituicyte and they may absorb lipoprotein derived from the limiting membrane of neurosecretory granules. When the hormone is released, the constituents of the membrane are liquefied and absorbed by pinocytosis, and lysosomes in the pituicytes partially digest them. Gradual accumulation of lipid droplets occurs.

The reticular pituicytes contain no lipid droplets normally. But few days after dehydration there is increase of lysosomes and small lipid droplets are noted in large lysosomes (Kurosumi, 1974). Normally reticular pituicytes are lesser than the protoplasmic pituicytes. Reticular pituicytes are engaged in phagocytic process involving degenerated neurosecretory axons and endings. Phagosomes are vacuoles containing the ingested material and after fusion with primary lysosomes, secondary lysosomes are formed. Regarding the other function of the pituicytes it is believed by some authors that they have an important role in the release of the neurosecretory material from the posterior lobe. The posterior pituitary hormones are produced in the supraoptic and paraventricular nuclei, and not in the pituicytes. It is also believed that pituicytes isolate the biologically active posterior pituitary hormones from the stainable component and they also help in the discharge of hormones into the blood vessels. Pituicytes may also play a role in ionic exchange at vessel walls or in chemical transformations on an enzymatic basis. Kurosumi (1974) doubted the function of impulse conduction by the pituicyte though there is clear evidence of axo-pituicytic contact. In the frog pituitary there are synapse-like contacts between nerve endings and pituicytes. Considerable thickening of the axolemma is noted at the contact place. Small clear vesicles are also accumulated on the thickened plasma membrane. Intersynaptic filaments occupy the intercellular



space in the typical synaptic clefts. Glycogen particles and mitochondria have been found in the nerve endings. This feature has rarely been observed in the posterior pituitary of the rat and synaptic vesicles are not accumulated on the thickened plasma membrane (Kurosumi, 1971).

### *Mammalian median eminence*

Rodriguez(1972) studied the comparative and functional morphology of the median eminence. The main structures are the ependymal and glial cells, nerve fibres and blood vessels. The median eminence can be divided into different regions. The *internal* or *neural region* contains ependymal lining, nerve tracts running towards the neural lobe, and long subependymal loops of the primary plexus of the hypothalamo-adenohypophysial portal system. In the *external* or *neurohaemal region* there are short loops of the portal system, different types of nerve endings, ependymal processes and glial cells. In higher mammals, including man, rostral extension of the median eminence is seen. Rodriguez considers this as a third region or *rostral region* of the mammalian median eminence.

Below the ependymal layer and in the subependymal region of the median eminence two neurosecretory tracts are situated, one comes from the supraoptic nucleus and the other from the paraventricular nucleus. One is *vasopressinergic* and the other one is *oxytocinergic*. No Cajal-positive fibres have been noted to end in the external region of the median eminence of the rat. Silver chromate method of Golgi revealed a rich plexus of nerve fibres in this location. This is due to a substance or a group of substances contained in the nerve and ependymal fibres.

Several types of median eminence nerve fibres could not be distinguished clearly in mammals as it could be in amphibians. Most of the endings in mammals contain clear and granulated vesicles smaller than 100 nm. According to Fuxe and Hokfelt(1967) majority of the nerve terminals of the median eminence contain monoamines and a small number of fibres contain releasing and inhibiting factors. Rodriguez thinks that most or perhaps all the terminals of the median eminence contain both, a monoamine, and a releasing or an inhibiting factor.

Short capillary loops are situated in the external region of the median eminence. The short loops and the processes of their perivascular basement membrane have contacts with nerve terminals, glial cells and ependymal processes. In the space surrounding the short loops there are only a few connective tissue cells.

The long capillary loops have been studied by Nowakowski(1951), Engelhardt(1956), Moll(1958), Enemar(1961), Lofgren(1961), and Duvernoy and Koritke(1964, 1968). The ascending and descending branches of the long loops are situated in the external median eminence and their subependymal portion is



parallel to the ependymal lining. The descending branch of the loop is thicker than the ascending one. These have been described in the chapter dealing with amphibian pituitary.

The capillary network in the rostral part of the median eminence has been observed in the monkey by Holmes(1967). Rodriguez(1972) observed them in the cow median eminence. This particular formation is an important site for release of neurosecretory substances in the vessels. In this location there are connections between ependyma and portal vessels and thus it is a site for local synthesis of secretory products. Large number of mast cells are seen and they should be considered regarding the synthesis and storage of serotonin. This vascular formation consists of complex network of intercommunicating blood vessels in the cow.

There are two types of ependymal cells: short and long. The processes end on the short and long loops of the primary plexus of hypothalamo-adenohypophysial portal system. The ultrastructural characteristics have been discussed in the chapter dealing with amphibian pituitary.

#### *Neurosecretory granules in the median eminence*

Ishii (1972) classified the neurosecretory granules in the median eminence of the horse. Kobayashi *et al.*(1970) reviewed the ultrastructural studies of the hypothalamic median eminence. Different types of hormonal substances are stored in the granules or electrondense vesicles. They are monoamines, neurohypophysial hormones, and adenyohypophysiotrophic hormones. Ishii (1972) reported in tabular form the average diameters of different types of electrondense vesicles of median eminence as observed by different investigators in various vertebrates. The classification was based on the different types of electrondense vesicles e.g. one or two groups of electrondense vesicles or three groups of the same or four groups of the same.

The axons of the equine median eminence were classified into A1, A2, B1, B2, and C. Group A1 was the smallest population having the modal diameter between 90 and 100 nm and the median of the diameter at about 94 nm. Group A2 has two types of vesicles. One has the mode at 110 nm and the other at 120 nm, in the same axon. Group B1 has vesicles whose median and mode coincide at 130 nm. Group B2 axon contains vesicles of composite population of two types with different modal diameters (about 140 and 155 nm). The vesicles of group C axons have the modal diameter at about 170 nm. Their median was also close to 170 nm; but the vesicles of this group may be of composite population.

*Two-group classification*—If the electrondense vesicles of the median eminence were to be divided into two groups, then the vesicles in both groups A1



and A2 axons are to be included in the category of small vesicles, and those of groups B and C axons in the category of large vesicles (Ishii, 1972). Most of the authors of two-group classification observed small vesicles only in the median eminence and the large vesicles were noted in the inner layer of the median eminence and the pars nervosa.

### *Three-group classification*

Most of the investigators in this group observed small and medium sized vesicles in the median eminence but not in the pars nervosa. Zambrano and De Robertis (1968) found medium and large sized vesicles for the axons of the pars nervosa. Ishii thinks that groups A1 and A2 vesicles correspond to small and medium sized vesicles of most workers. Group B and C vesicles correspond to large vesicles of most authors or to medium and large size vesicles of Zambrano and De Robertis (1968).

### *Four-group classification*

In the equine median eminence Ishii observed four kinds of electrondense vesicles with modal diameters of 100, 120, 135 and 155 or 165 nm (Kobayashi *et al.*, 1970; Ishii, 1970). They correspond to groups A1, A2, B1, and B2 or C respectively.

Two types of vesicles in the same axon (80 and 130 nm) were reported by Rodriguez (1969), and Ishii (1972).

Noradrenaline was concentrated in group A1 vesicle and dopamine is contained in the vesicles of group C axon. Gonadotrophin releasing hormone was contained in group A2 vesicles. Corticotrophin releasing hormone is contained in group B1 axon (Peczely and Calas, 1970; Akmayev *et al.*, 1967; Kobayashi *et al.*, 1970; Ishii *et al.*, 1969; and Mulder, 1970).

### *Neurosecretion*

Kurosumi (1974) classified the neurosecretory systems of higher vertebrates into :

- (a) Hypothalamo-hypophysial system which produces posterior pituitary hormones e.g. oxytocin and vasopressin (ADH) and
- (b) Hypothalamo-infundibular system which produces the releasing and inhibitory factors (hormones) e.g. STH—RH, TRH, CRH, LH—RH, FSH—RH and PIH.

In the zona interna these two systems cross each other at right angles. The neurosecretory granules of the hypothalamo-hypophysial system are 200-300 nm in diameter and those of the hypothalamo-infundibular system are 100 nm in diameter. In the zona externa of the rat the axons and nerve terminals contain



a large number of synaptic vesicles, small dense granules and many mitochondria.

The ultrastructure of the neurosecretory cells in the nucleus supraopticus and paraventricularis of the rat shows well developed Golgi apparatus and rough endoplasmic reticulum. There are small dense granules of about 200 nm in diameter in the region of the Golgi apparatus. The neurosecretory material is synthesized on the ribosomes at the outer surface of the rough endoplasmic reticulum which corresponds to the Nissl substance of light microscope. The proteinaceous substance collects in the cavity of the rough endoplasmic reticulum and subsequently transported to the Golgi apparatus and the elementary granules of the neurosecretory substance are formed.

Neurosecretory cells are mostly spherical or oval in shape with round nuclei. There is clear cytoplasm with free or membrane-bound ribosomes. Some irregular, small neurosecretory cells possess dark cytoplasm and these are known as *dark neurosecretory cells*. The outline of the shrunken nucleus is wavy but the nucleolus looks normal. Some neurosecretory granules are found. The Golgi apparatus is not so well developed. The cytoplasm contains plenty of free ribosomes. Lysosomes increase in number in these cells (Kurosumi, 1974). He thinks that the water content of this cell is small and it shows probably a pathological degeneration. This is also suggested by the increase in the number of lysosomes in these cells.

Direct communication between the smooth surfaced membrane of the Golgi apparatus and the rough-surfaced endoplasmic reticulum can be found in the neurosecretory cells of the supraoptic nucleus of the rat. Thus the transport of immature neurosecretory substance from the cavity of the rough-surfaced endoplasmic reticulum to the Golgi apparatus can occur and maturity of the granules can take place in the Golgi cisternae (Kurosumi, 1974). Transport by budding of small vesicles from the rough-surfaced endoplasmic reticulum is also another possibility. Mature, dense granules are 200 nm in diameter. Between the limiting membrane and dense core of the granule there is a narrow clear space. Kurosumi observed this clear space to increase in immature granules and in those granules at the nerve terminals of the posterior pituitary before they are discharged, because during the process of discharge the periphery of the dense core is dissolved. According to Kurosumi there is no variation in the size of the granules in the posterior pituitary when compared to that at the neurosecretory cells.

There are axosomatic synapses on the neurosecretory cell bodies. The synaptic vesicles (50 nm) and mitochondria are located at the endings. A little larger cored vesicles are found in some. Thickening of the plasma membranes occurs at the contact site of the nerve ending with the surface of the neurosecretory cell like a desmosome. At the presynaptic part many synaptic vesicles are found. Cored vesicles (100 nm diameter) are always seen. They are located at a distance from the thickened plasma membrane. Some authors think them to be



monoaminergic. There is *subsynaptic sac* (subsurface cistern) and subjacent rough endoplasmic reticulum. A dilated cistern of the rough endoplasmic reticulum is located under the smooth subsurface cistern. Its surface facing the smooth subsurface cistern is also smooth but the opposite side facing the interior of the cell is rough and studded with ribosomes (Kurosumi, 1974). Kurosumi thinks that a close approximation between rough endoplasmic reticulum and the subsurface cistern probably functions in conducting an information regarding synthesis of the protein. Heaps of synaptic vesicles could not be seen by him just outside the subsurface cistern.

In the neurosecretory terminals at the perivascular areas in the posterior pituitary there are peripheral neurosecretory granules (150 nm—200 nm). Neurotubules form a ring-like pattern at the ending and there are also microvesicles (50 nm)—which are located centrally. The empty vesicles are remnants after the release of the granules (hormone) by the method of molecular dispersion (Kurosumi, 1974). Kurosumi found morphological similarity between the synaptic vesicles in the ordinary nerve ending and microvesicles in the neurosecretory terminal. These microvesicles may contain acetylcholine or some other transmitter substance.

Neurosecretory material is discharged from the terminals by (a) *molecular dispersion* as suggested by Fujita and Hartmann (1961) which is same as Kurosumi's type IV or diacrine mechanism: and by (b) *exocytosis*. The mechanism of exocytosis is same as Kurosumi's type IV or eruptocrine mechanism. Kurosumi could not find exocytosis in normal or dehydrated rats. At the neurosecretory axons flattened sacs were found. These sacs may be membranes which covered the neurosecretory granules. With the release of the hormone in the posterior pituitary the limiting membrane of the neurosecretory granules forms these flattened sacs. These flattened sacs ultimately get fragmented with the formation of microvesicles (50 nm in diameter). Some of them may be synaptic vesicles which contain acetylcholine or other transmitter substances (Kurosumi, 1974).

#### *Further observations on mammalian median eminence*

There are terminations of hypothalamic nerve fibres on the capillaries of the hypophysial portal vessels in the median eminence (Bargmann, 1953; Szentagothai, 1964; Rinne, 1966; and Fuxe, 1964). Large number of granular and/or agranular vesicles have been noted in the perivascular nerve endings (Rinne, 1966; Monroe, 1967; and Rodriguez, 1969). These inclusions may be the storage sites of monoamines. Close correlation between the vesicles and the monoamines was observed by many but Mazzuca (1965), and Monroe (1967) did not observe the same. Arko *et al.* (1962, 1963) noted the appearance of many aldehyde-fuchsin-staining nerve fibres in the outer layer of the median eminence of the rat after bilateral adrenalectomy. This has been confirmed by many workers (Bock and aus der Muhlen, 1968; Goebel, 1968; Stohr, 1969; Bock and v. Forstner, 1969; and Bock *et al.* 1969).



Rinne(1970) concluded that the granular vesicles which are rendered visible by glutaraldehyde and osmium in the median eminence of the rat do not contain dopamine. In the median eminence however, small granular vesicles are engaged in the storage or metabolism of noradrenaline.

Akmayev *et al.* (1967) noted plenty of large empty vesicles in the hypothalamic median eminence (Zona palisadica) of the albino rats 10 to 17 days after bilateral adrenalectomy. Rinne (1970) further states that the observations after bilateral adrenalectomy support the view that the aldehyde-fuchsin-staining substance and the granular vesicles are engaged in the neurohumoral control of corticotrophin secretion. However, there is no evidence in favour of dopamine playing a role in this process. "The large granular vesicles may act as the storage site of corticotrophin—releasing factor and may be the subcellular structures corresponding to the aldehyde-fuchsin-staining substance in the median eminence". Rinne (1972) has confirmed this finding. In the normal rat occasional aldehyde-fuchsin-positive, very fine and beaded nerve fibres could be detected in the outer layer of the median eminence and they proceed towards the hypophyseal portal capillaries. Plenty of such nerve fibres could be detected in the outer layer of the median eminence after bilateral adrenalectomy in the rats. There were no clear changes in the number and intensity of the yellow-green fluorescent arcuate cell bodies by histochemical studies of primary catecholamines. After adrenalectomy, the intensity of the fluorescence in the median eminence remained unchanged. "Electron microscopic examination showed that in a normal rat the perivascular nerve endings in the median eminence contained a great number of inclusions, both agranular vesicles and typical granular dense-core vesicles. After bilateral adrenalectomy the total number of granular vesicles decreased significantly. This decrease took place in the numbers of small granular vesicles. In contrast, the number of large granular vesicles about 1,300-1,600 Å in size increased significantly. Correspondingly, the proportion of large agranular vesicles had risen somewhat." A new population of large granular vesicles could be seen which did not exist in the control material.

Wittkowski *et al.* (1970) showed that the Gomori-positive granules of the zona externa and the neurosecretory substances of the supraoptico-hypophyseal system in the zona interna are made up of small, round, elementary granules. Vernikos-Danellis (1965) noted that there was increase of CRF activity in the rat median eminence after bilateral adrenalectomy and reduction of CRF activity in the median eminence of female rats follows hydrocortisone treatment. Wittkowski and Bock(1972) said that the chromalum-gallocyanine method of Bock(1966) is good for combined light and electron microscopic visualization and assessment of Gomori-positive material. The disadvantage of the method is because of the poor preservation of tissue. In such preparations cell nuclei and Gomori-positive structures could be distinguished with the help of electron microscope. Membranes were not preserved. With osmium staining, granule content and the granule membrane could be visualized and the intervening space



between the granule and the membrane could also be noted but with gallocyanine method only the granule could be visualized. Therefore, while investigating the same type of granules, the diameter is expected to be 200-400 Å larger in osmium treated material than with the gallocyanine method. In the normal rat the zona externa contains small aggregations of Gomori-positive elementary granules very near the capillaries of the portal plexus. The number of 400 Å elementary granules is three times less in comparison to the 1,100 Å granules of the zona interna. In osmium preparations they should correspond to 700 Å in the zona externa and 1,500 Å in the zona interna respectively. The granule aggregations are of 0.3 to 0.4 µm in diameter in the zona externa. The Gomori-positive elementary granules of the zona externa lie within nerve fibres as in zona interna. After bilateral adrenalectomy these granules in the zona externa increase in number and in diameter (800 Å) in seven days. At three weeks the size is 900 Å. The granule aggregations increase to 1 µm. When substitution therapy with hydrocortisone from the day of operation onwards is done, the relationship between corticoid balance and amount and size of the elementary granules can be clearly demonstrated. The increase in number and size is due to synthesis and storage of CRF. The decrease after adrenalectomy and substitution therapy is due to an inhibitory effect of the therapy. "The paradoxical effect of hydrocortisone treatment when beginning on the 14th day p.o. (post operative), which brings about an enhanced increase of the *Gomori-positive* substance in the zona externa, may be explained in the following way: during the 14 days p.o. an enhanced synthesis and storage of CRF takes place; application of hydrocortisone after this period probably only inhibits the release of CRF and does not immediately stop CRF synthesis".



## CHAPTER 5

### CONTROL OF THE PITUITARY GLAND IN MAMMALS

The mechanisms involving the control have been reviewed by Roy(1976) in his book, "Neuroendocrinological studies in stress—Experimental surgical observations in vertebrates and invertebrates" along with his own experimental observations. They comprised stimulation and lesion experiments of the hypothalamus and extrahypothalamic areas, pituitary stalk section experiments, grafting experiments, hypophysectomy (partial and total), corticotrophin releasing factors and hormone, pituitary autografting in the hypophysiotrophic area (fig. 5.1).

In this Laboratory my student Misra(1971) studied the role of hypothalamic and extra-hypothalamic neural structures in the regulation of ACTH release by stimulation and lesion experiments. Hippocampus, basolateral amygdaloid nucleus and dorsal septum are inhibitory centres to the pituitary-adrenal axis; whereas, corticomedial amygdaloid nucleus, ventral septum and medial midbrain reticular formation are facilitatory areas to the said axis. The insula though facilitatory, but its lesion has not affected the axis. It is interesting to note that stress of burn following lesion of these areas stimulated the pituitary adrenal function in all the instances.

Allen *et al.*(1977) reviewed the experimental models for the study of pituitary function. The authors prepared and evaluated the isolated pituitary in rats. They have described a surgical procedure which is a modification of Smith's parapharyngeal approach for hypophysectomy. After separation of the stalk, a metal barrier measuring approximately 2×4mm is inserted between the cut ends.

Changes in the pituitary after stalk section were studied by light and electron microscopes. Central necrosis in the adeno-hypophysis surrounded by surviving cells was found. The viable cells were adjacent to the dura and infundibular process. The infarcted area contracted after fourteen days of surgery and this was surrounded by viable pituitary cells. PAS + gonadotrophs were found in the peripheral areas after seven days and they increased after fourteen days. Ultra-structurally cells surrounding the infarcted area were vacuolated and contained cytoplasmic inclusions and a few granules. These cells usually lacked plasma membranes. Prolactin cells looked rather normal but mature granules were not found. In the peripheral part of the pituitary of shortterm stalk-sectioned females the FSH cells were well granulated. FSH cells were more than LH cell and they looked somewhat anoxic. In stalksectioned rats there were no adeno-hypophysial castration cells after ovariectomy. Gonadotrophs had similar pictures as noted in stalksectioned animals.



After stalksection ovarian weights, uterine weights, and adrenal weights diminished. After castration alone there was decrease in uterine weights. Daily vaginal smears in the non-castrate, stalksectioned animals showed a constant diestrus pattern.

The pituitary has been permanently isolated in these experiments as demonstrated by very low FSH and LH levels and high prolactin levels in noncastrate and ovariectomized animals sacrificed at various intervals after stalk section. When LHRH is administered subcutaneously, significant rise of LH and FSH occurs which proves the presence of significant numbers of responsive gonadotrophs, although the LH response has been noted after intraarterial injection of LHRH, and FSH response is not. In this experimental model interaction between estradiol and LHRH at the pituitary level has also been demonstrated. LH levels greatly increased by prior administration of estradiol benzoate.

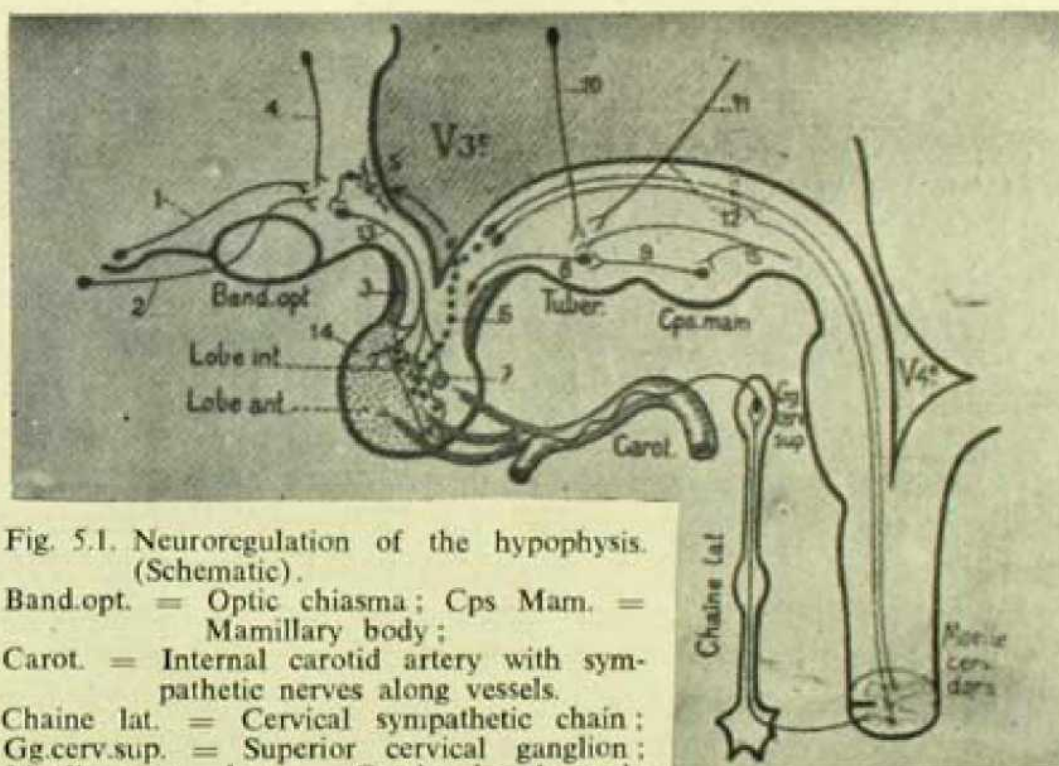


Fig. 5.1. Neuroregulation of the hypophysis. (Schematic).

Band. opt. = Optic chiasma; Cps Mam. = Mamillary body;

Carot. = Internal carotid artery with sympathetic nerves along vessels.

Chaine lat. = Cervical sympathetic chain;

Gg. cerv. sup. = Superior cervical ganglion;

Moelle cerv.-dors. = Cervico-dorsal cord;

1 = Amygdalo-tangential tract (olfacto-hypophysial reflex path); 2 = Retino-tangential tract (Optico-hypophysial reflex path); 3 = Pars tuberalis; 4 = Strio-hypothalamic path (Influence of corpus striatum and globus pallidus on the hypophysis); 5 = Subependymal nerve plexus; 6 = Path of neurocrinie from the hypophysis to the hypothalamus; 7 = Glandular islet in the posterior lobe; 8 = Tractus tubero-hypophysius; 9 = Tractus mamillo-hypothalamicus (Olfacto and sensitivo-hypophysial reflex); 10 = Talamo-hypothalamic path; 11 = Cortico-hypothalamic path; 12 = Descending hypothalamic path; 13 = Tractus hypothalamo-hypophysius; 14 = Zone of transition; 15 = Central sensory path (Sensitivo-hypophysial reflex).

From G. Roussy and M. Mosinger (1946).

Courtesy of Masson et Cie., Paris.

### *Releasing factors of the hypothalamus*

Guillemin (1976) discussed the physiological and clinical significance of hypothalamic and extrahypothalamic brain peptides.



Burgus *et al.* (1969) isolated the first of the hypothalamic hypophysiotrophic peptides, the thyrotrophin releasing factor (TRF) from sheep hypothalami in December 1968. The hypothalamus regulates the functions of the thyroid gland through the pituitary by this molecule. Schally *et al.* (1970) described the structure of the porcine TRF in 1969. Guillemin's group established the chemical structure of ovine TRF as a simple tripeptide pGlu-His-Pro-NH<sub>2</sub>. Schally's group found this structure to be identical. The synthetic replicate was highly potent in all vertebrate species and specially in man. TRF stimulates the secretion of prolactin as well as that of thyrotrophin (TSH) (Tashjian). The stimulating effect on prolactin secretion is variable in different species but it happens in man.

Schally's group in 1971 isolated the hypothalamic hypophysiotrophic peptide hormone which controls the secretion of LH and FSH. They proposed the structure of the porcine luteinizing hormone releasing hormone (LHRH) as that of the decapeptide pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>. Guillemin's group later on confirmed the structure. The synthetic hormone is now widely used in clinical medicine and veterinary medicine for stimulating ovulation and this is now widely used in the treatment of certain types of infertility in men and women. The authors (Guillemin's group) isolated in 1972 synthetic analogs of LHRH having antagonistic activity against LHRH. This antagonistic action may be utilized for fertility control.

Celis *et al.* (1971) found the structure of MIF to be a tripeptide.

A tetradecapeptide was isolated from the ovine hypothalami by Guillemin's group (Brazeau *et al.* (1973). This peptide inhibited the release of growth hormone from the anterior pituitary and is known as somatostatin. Vale *et al.* (1975) noted that somatostatin inhibits TRH-stimulated release of TSH and prolactin release is inhibited in some situations. Koerker *et al.* (1974) found this peptide to inhibit the release of insulin and glucagon from the pancreas. Somatostatin has been found in discrete cells of the endocrine pancreas (D cells) and also in other tissues of the gastrointestinal tract. These cells apparently derived from the neural crest during ontogeny (Guillemin, 1976). Somatostatin is manufactured in these peripheral cells. These peptides have been called by Guillemin as *Cybernin*. All these releasing factors (peptides) have also direct effect in the central nervous system. TRF may have an antidepressive effect in some humans (Kastin *et al.*, 1972; Prange *et al.*, 1972; Wilson *et al.*, 1973). LHRH stimulates mating behaviour in rats (Moss and McCann, 1973; Taurog *et al.*, 1974). TRF is present in human CSF (Oliver *et al.*, 1974) and in the CSF of rats (Joseph *et al.*, 1975).



## CHAPTER 6

### THE PITUITARY GLAND OF MAMMALS

Costoff(1973) summarized the current views of rat anterior pituitary cell types and their properties. Many of the cell types have also been identified by fluorescent-antibody technique, autoradiography and immunoelectron microscopy.

#### *Somatotrophs :*

Somatotrophin is secreted by acidophil somatotroph. These cells have been characterized in acromegaly and tumors. They are absent in the dwarf mice. Dwarf mice are not only deficient in growth hormone; evidences of deficiency in thyrotrophic, gonadotrophic, and corticotrophic function also exist in them. Typical thyrotrophs and gonadotrophs were found in the anterior pituitary of the dwarf mice but the thyrotrophs, in particular, were very scanty in number and the thyrotrophin content was only one tenth of the normal (Ortman,1956). Though there are multiple deficiencies both hormonally and structurally, somatotrophin and acidophil granules are the only components in each class that are totally deficient (Purves,1966).

In the rat two types of acidophil cells have been described by Purves and Griesbach(1952). In thyroid insufficiency the cell responsible for the release of growth hormone was found by them to be degranulated. These cells (somatotrophs) were not affected by estrogen treatment or castration but the LTH cells were found to be affected. Somatotrophic activity disappeared after loss of granules from the  $\alpha$ -cells (Knigge, 1958). Griesbach *et al.*, (1963) observed that previously degranulated somatotrophs in thyroidectomized rats regranulated after administration of potassium iodide. Hydrocortisone treatment in similar situation gave rise to regranulation of the somatotrophs to 60% of normal after 14 days (Meyer and Evans, 1964).

Vanha-Perttula(1966) identified the STH cells as most abundant chromophilic cell type in the pituitary gland of the control rats and immature rats. They were round or oval orangeophilic cells when stained with methods including orange G. With azocarmine-orange G method the cell took up ochre or light brown colour. It is uniformly distributed in the anterior pituitary gland and is also situated in small groups along the sinusoids. STH cells in immature rats are plenty in number and with testosterone treatment they had increased affinity for orange G. With methylthiouracil feeding the cells stained less and there were only some ochre-stained granules in the cytoplasm and the cells looked vesicular. They were deeply red stained with acid fuchsin-aniline blue method but could not be differentiated from the lactotrophic cells in pregnant and lactating female rats.



Staining reactions of different cell types after various staining methods (Vanha-Perttula, 1966)

Cell type	PAS -orange G	Aldehyde -fuchsin	Alcian blue		Tetra- chrome	Acid fuch- sin-aniline blue	Aldehyde -thionin	Azocarmine orange G	Nomen- clature (Herlant)	Localization
			pH 3.0	pH 0.2						
STH	orange	unstained	orange	orange	orange	red	orange	ochre	alpha	in small groups
LTH	orange	unstained	orange	pale red	brick red	red	orange	carmine	eta	separately or in palissades
ACTH	orange granules	unstained	unstained	unstained	violet granules	violet granules	orange granules	violet granules	epsilon	separately or in palissades
TSH	red	red	green	green	dark blue	blue	dark blue	dark blue	delta	central
LH	red	unstained	light green	red	light blue	violet	red	light blue	gamma	central
FSH	red	unstained	violet	violet	light blue	violet	light blue	light blue or violet	beta	peripheral
Endo- thelium	red	pink	red	unstained	blue	unstained	blue	blue		
Peri- cytes	red	pink	red	pink	blue	blue	blue	blue		



They could, however, be differentiated by the tetrachrome method in addition to the azocarmine—orange G method. With the former method the STH cells were orange stained but in this method the concomitant aniline blue appreciably overstains these cells. So the latter method is more specific for STH cells.

#### Staining characteristics

Staining method	Colour of granules	Authors
Orange G	Orange	Dawson (1954, 1963)
Tetrachrome method	Orange	Pasteels and Herlant (1962)
Azofuchsin, orange G in phosphotungstic acid and Wool green S	Orange	Brookes (1967, 1968)

STH cells have been distinguished by immunofluorescence method in the rat, mouse and domestic animals by Rumke and Ladiges (1965) and Shiino and Rennels (1966). Similar method has been applied in man by Grumbach (1962) and Leznoff *et al.* (1960). Baker *et al.* (1969) and Baker (1970) could distinguish two types of acidophils in the rat by peroxidase-labeled antibody methods of Nakane and Pierce (1967). Nakane (1970) identified the somatotroph at the electron microscope level by using peroxidase-labeled antibody method.

#### Electron microscopic observations

Farquhar and Rinehart (1954a,b) and Yoshimura and Harumiya (1965) noted degranulation of somatotrophs after thyroidectomy or castration. Rinehart and Farquhar (1953) and Hedinger and Farquhar (1957) said that the somatotrophs could be easily identified and were plenty in number in the rat pituitary gland (fig. 6.1). The secretory granules were about 350m $\mu$  in diameter. There were Golgi complex, mitochondria with interrupted cristae and a well developed endoplasmic reticulum. Rennels and McNitt (1958) noted the absence of somatotrophs in dwarf mice pituitaries. Hypertrophy and hyperplasia of STH cells in a diabetic strain of mice were observed by Yamada *et al.* (1967).

Couch *et al.* (1969) injected purified growth hormone releasing factor (GRF) into rats. They examined the pituitaries at different times ultrastructurally and noted the release of plenty of granules by *exocytosis* from the somatotrophs.

By radioimmunoassays Birge *et al.* (1967) noted more somatotrophin in the male rat pituitary than in female rats. Similar observation came from Costoff (1973). The peripheral part of the gland has more STH activity or preponderance of more STH cells in the periphery of the gland (Smith and Smith, 1923; Rinehart and Farquhar, 1953; Herlant, 1964; Costoff, 1973).

Costoff (1973) studied the ultrastructures of somatotrophs in intact controls and after other treatments in rats. This is the most preponderant cell type : more



in the male than in the female rat. Cells are peripherally situated. The size of the cells and the granules is bigger in the male rat than that in the female. Cells

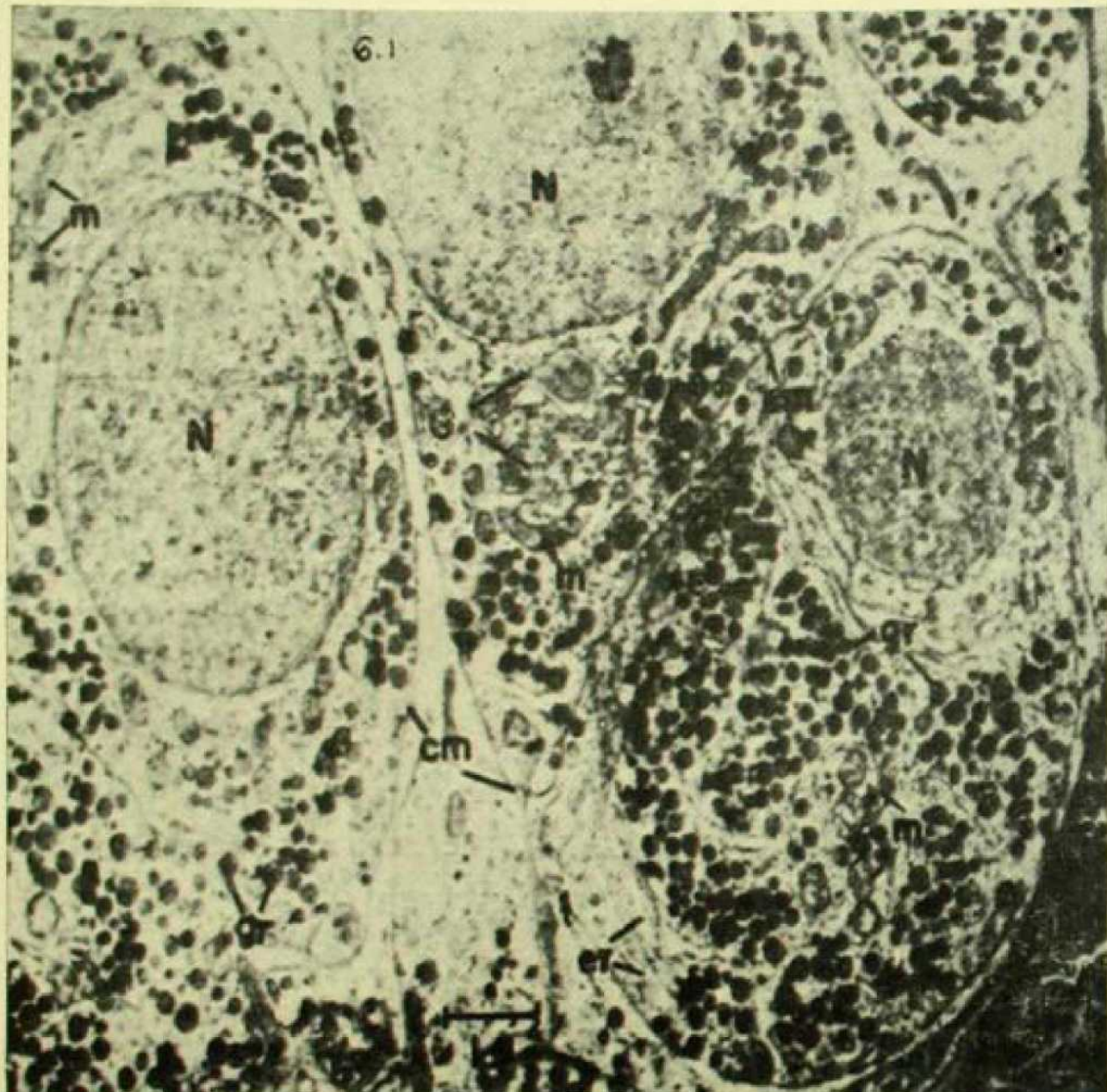


Fig. 6.1. Electron micrograph showing cells from the anterior pituitary of a young adult male rat. Three acidophils of the type which are thought to be responsible for the production of growth hormone (Hedinger and Farquhar, 1957; Farquhar and Rinehart, 1954a) occupy most of the field. Their nuclei (N) are indicated.

This type of acidophil is characteristically rounded or ovoid in shape, and the cells typically are arranged in groups, as shown here. In electron micrographs the most distinctive feature is their content of variable numbers of dense, ovoid secretory granules (gr) of a characteristic size (ca. 350 m $\mu$  maximal diameter). Large numbers of secretory granules are present in this field. The cell membranes (cm) can be clearly seen separating the cytoplasm of one cell from that of another. Mitochondria (m), endoplasmic reticulum (er), and Golgi material (G) may also be distinguished.  $\times 10,300$ . Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).

are found to be away from the capillaries and are of medium size, oval in shape with plenty of large, round, dense secretory granules. The granules vary from 150-400 m $\mu$  in diameter with a mean of 235m $\mu$ . The nucleus is round and



eccentric in position. The extensive rough endoplasmic reticulum is situated at one pole of the cell. On the membrane surface the ribosomes are not evenly distributed and frequently the smooth areas of endoplasmic reticulum have been noted to face the Golgi apparatus. The Golgi complexes are either one or more and are situated near the nucleus. They consist of flattened or dilated sacs and tubules. An active somatotroph is characterized by a Golgi system having vesicles of 100m $\mu$  in diameter, flattened vacuoles arranged in parallel, and secretory granules in different stages of formation. Multivesicular bodies and lysosomes have been noted in and around the Golgi region. Mitochondria are round or rod-shaped. Centrioles are situated either near the cell surface or near the Golgi region. Sometimes a cilium was noted and this was associated with centrioles very near the cell surface.

Costoff observed two types of somatotrophs in the adult male pituitary.

*Active type*: It presents with well-developed rough endoplasmic reticulum, hypertrophied Golgi apparatus and a few granules at the periphery of the cell. Release of granular material could be seen at the cell membrane. The location of these active cells is the central part of the gland.

*Quiescent somatotroph*: This is smaller and is of storage-type. Cells are peripherally situated in the adult pituitary gland and this area is poor in vascular supply. Plenty of granules in this type of cell often obscured the scanty Golgi apparatus and endoplasmic reticulum.

*Adrenalectomy and somatotrophs*:

Definite change occurs after 14 days with plenty of lysosomes and decrease of granulations. One-third of the somatotrophs appeared degenerating after 30 days of adrenalectomy. At this period the cells had few granules, a faint endoplasmic reticulum, free ribosomes, an involuted Golgi complex, swollen mitochondria and the cytoplasm was small. The granules were smaller. There was an increase in cilia in some somatotrophs. Costoff said that these degenerating somatotrophs could be designated as chromophobes by light microscopy.

Costoff (1973) also studied changes in these cells after *Propylthiouracil* treatment when there was increase in lysosomes with degranulation. The cells were of *storage-type*.

Degranulation occurs long after *castration* with an increase of lysosomes. *Adrenalectomy + castration* showed degranulation with swollen mitochondria. Similar features were noted by him after *metopirone*, *amphenone*, *hydrocortisone* and *dexamethasone* treatment. Peripheral somatotrophs showed maximum degranulation. *Storage-type* STH cells were noted after *ACTH* and  $\alpha$ -*ethyltryptamine* treatment.

After *long-term thyroidectomy* there was degranulation of somatotrophs (Purves and Griesbach, 1952; Knigge, 1958; and Farquhar and Rinehart, 1954b). *Castration* in rats showed similar features (Severinghaus, 1937; and Farquhar and Rinehart, 1954a).





Lysosomes could control the production of STH or LTH depending on the demand by phagocytosis of the hormone granules

66.6% of the somatotrophic activity could be found by Costoff in the granule FE1LSP pellet, whereas 19.3% was found in the soluble SA zone. The granules in the FE1LSP pellet had a mean diameter of 242m $\mu$  whereas the granules in the somatotrophs had a mean of 240m $\mu$ . Most of the STH activity should be in the FE1LSP pellet.

### *Thyrotrophs :*

Thyroidectomy gave rise to degranulation of acidophils and hypertrophy of certain basophils. Thus there is a problem regarding the cell type which produces TSH.

### *Production of TSH*

*by*

Acidophils

Basophils

Severinghaus  
(1937)

Zeckwer *et al.* (1935)

Griesbach (1941)

Griesbach and Purves (1945)

Vanha-Perttula (1966) observed that with aldehyde fuchsin only certain central polygonal cells of the rat pituitary are stained red. With aldehyde-thionin method the same cells stained dark-blue. The cells were green with alcian blue at pH0.2. At pH3.0 other cells had some weaker affinity for the dye. These AF-positive cells are large cells containing fine granulations and they do not intimately border the sinusoids but contact these through their branches. Sometimes these cells were arranged in grape-like distribution along a sinusoid. After methylthiouracil feeding these cells increased in number with the formation of small irregular groups. With thyroxine treatment TSH cells decreased in number and they were shrunken in appearance. These cells are PAS-positive and using acid fuchsin-aniline blue as well as tetrachrome methods, the intensity of the blue stain in TSH cells was stronger than in any other *mucoid* cells.

In all species of animals studied by Pearse (1949) PAS-positive material was present in basophils and certain basophilic chromophobes. Purves and Griesbach (1951a,b) differentiated two types of basophils by PAS staining. Polyhedral cells were situated in the center of the gland and they were considered as TSH-cells. These cells hypertrophied in thyroidectomized animals and they decreased in size after administration of thyroxine in intact rats. Basophil cells remaining unaltered in the above circumstances were considered by them to be gonadotrophs. These authors could also identify the thyrotrophs by aldehyde fuchsin(AF) stain of Gomori where the granules stained red. This was supported by Halmi (1952) and Dhoni and Tieze (1962). With the tetrachrome staining method Pasteels and Herlant (1963) observed the TSH granules to





stain dark blue. More intense PAS reaction of the TSH granules than that of the gonadotrophs was considered by Herlant to be due to more mucoproteinaceous material in TSH granules (Herlant, 1964).

By immunofluorescence technique TSH cells were identified by Greenspan and Hargadine (1965). By peroxidase-labeled antibody technique Nakane (1968) noted clustered TSH cells in the center of the gland. Vanha-Perttula (1966) noted fine granules in the AF-positive TSH cells. These cells were found in groups and bordered the capillaries. After thyroidectomy or PTU treatment increase in thyroidectomy cells was observed in comparison to TSH cells in the intact group of animals. Dingemans (1969) found that the number of LTH, STH and LH cells diminished after thyroidectomy and he thought that these cells could lead to the production of thyroidectomy cells. After treatment with PTU fewer granules were noted in TSH cells (Contopoulos *et al.*, (1958). D'Angelo (1961) observed increase in TSH by 300% in the blood but the same was found to be reduced by 95% in the pituitary gland. The inference is that there is a negative feedback of thyroxine on the pituitary or the hypothalamus and thereby inhibition of TSH release takes place and increase storage of TSH in the granules happen. Very little thyroxine is produced after thyroidectomy or PTU treatment. There is continuous secretion of TSH in blood with very small storage.

#### *Electron microscopic observations*

After thyroidectomy in mice there was dilated endoplasmic reticulum with decrease in granulation in the hypertrophied thyrotrophs (Barnes, 1963). With thyroxine administration there was regranulation of these cells. Potvliege (1970) observed that administration of PTU and estrogen in the rat led to *regular intact* TSH cells and lesser number of thyroidectomy cells and thus estrogen prevents the changes in TSH cells after treatment with PTU.

Farquhar and Rinehart (1954b) observed hypertrophy of cells responsible for TSH secretion in rats after thyroidectomy (fig. 6.2). Kurosuni and Oota (1966) could differentiate thyrotrophs from gonadotrophs and corticotrophs.

#### *Costoff's observations (1973) :*

1 to 2% of cells are thyrotrophs in the rat pituitary gland. These are the smallest type of cells except certain chromophobes and are situated in the mid-central and midlateral parts of the gland. These *angular* cells are situated on a capillary. The cells have little cytoplasm and plenty of granules are arranged around the periphery of the cell near the cell membrane. The diameter of the granules varies from 40-150m $\mu$  with a mean of 88m $\mu$ . The density of the granules in these cells is lesser in comparison to that of the acidophilic granules and the membranes adhere closely to the granules. Near the cell membrane the centrioles with cilia are sometimes observed. The endoplasmic reticulum is poorly developed and is usually vesicular in appearance. The Golgi apparatus with formation of granules in different stages is seen near the nucleus. Lysosomes are infrequent. "Mitochondria, ovoid or short rod-shaped bodies contain-



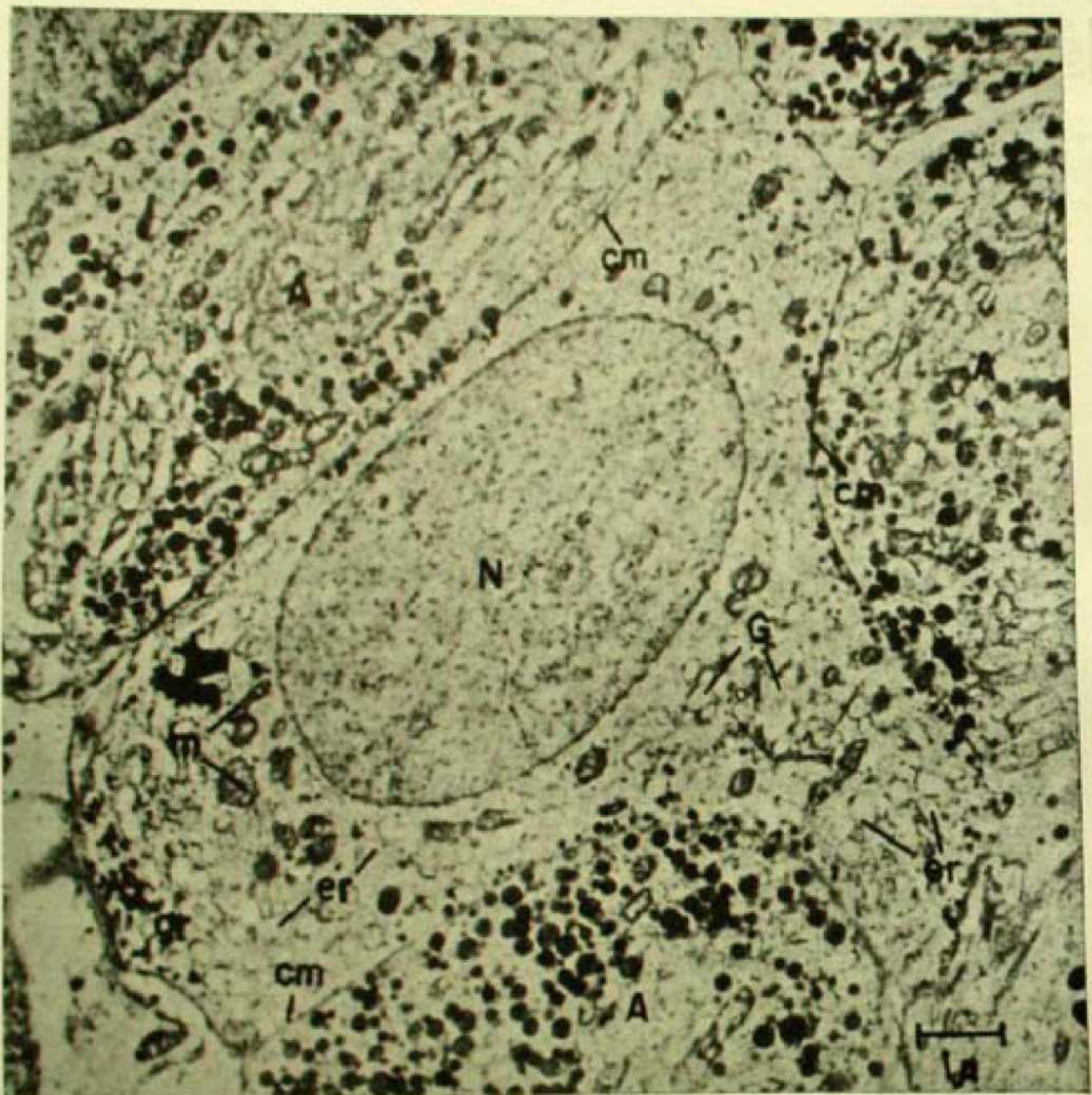


Fig. 6.2. Electron micrograph showing a thyrotroph from the anterior pituitary of a young adult male rat. The nucleus (N) and cell membranes (cm) are indicated. The irregular contour characteristic of thyrotrophs is illustrated in the angular shape of this cell.

In electron micrographs thyrotrophs can be distinguished by virtue of the size of their secretory granules which are smaller (maximal diameter ca.  $100m\mu$ ) than those in any other type of anterior lobe cell (Farquhar and Rinehart 1954b). In this cell the secretory granules are found, for the most part, lined up along the cell membrane.

As seen in this cell, the endoplasmic reticulum (er) of thyrotrophs is generally present in the form of small vesicular profiles with occasional elongated (cisternal) profiles. The mitochondria (m) usually occur in the form of short rods and show a background matrix which is much less dense than the internal matrix of gonadotroph mitochondria. A group of vacuoles of the Golgi complex (G) can also be distinguished in the cytoplasm of the thyrotroph.

The thyrotroph is virtually surrounded by acidophils of the growth hormone type. The cytoplasm of these cells is labeled A. The size of their secretory granules (maximal diameter ca.  $350m\mu$ ) can be contrasted with the smaller thyrotrophic granules  $\times 10,000$ . Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



ing interrupted cristae randomly oriented are not found in these cells". After daily administration of PTU in doses of 10-20mg to rats there was an increase of thyroidectomy cells (Bogdanove and Halmi,1953).

#### *PTU treatment (Costoff,1973)*

The angular thyrotrophs increase in size upto tenfold with an increase in number of these hypertrophied cells. Wide dilatation of the rough endoplasmic reticulum and fusion of the vesicles occurs and thus the vacuolated endoplasmic reticulum of the thyroidectomy cell, which is very characteristic, is formed. Golgi apparatus is hypertrophied with deficiency of granules and it is better defined. This is the picture of an actively secreting gland and without storage. Hypertrophy of mitochondria is found and they assume oval or spherical shape. The nuclei are indented with plenty of nucleoli. The few granules in these cells measure 40m $\mu$  more than those in untreated TSH cells.

Nakane(1970) and Baker and Yu(1971) used peroxidase labeled antibody technique and described the pattern of distribution and characters of TSH cells.

The electron micrograph of DIHSP pellet (Costoff, 1973) shows it to be free of contamination of other cell organelles and the granules had a mean diameter of 89m $\mu$  which favourably compares with the TSH cell granule from whole tissue where it is 85m $\mu$ . Measurement of the granules and bioassays prove that these cells are thyrotrophs.

#### *Mammotrophs :*

LTH cells are acidophils. They are carminophilic (Dawson, 1954), orangeophilic (Lacour, 1950; Sanders and Rennels, 1959), erythrosinophilic (Pasteels and Herlant,1962; Pasteels,1963), sulfhydryl and/or disulfide positive (Barnett *et al.*, 1961).

These cells are more active during pregnancy and lactation in the rat (Dawson, 1963). The granules are depleted during suckling of the litter and there is a rapid fall in the lactotrophic hormone content of the pituitary gland (Grosvenor and Turner, 1958). That LTH is localized in acidophils of the rat pituitary gland has been confirmed by immunofluorescence method (Emmart *et al.*, 1963; Rumke and Ladiges,1965). Emmart *et al.* (1965) noted them in a prolactin tumor. Shiino and Rennels(1966) noted these cells in the rabbit. By the same technique Nayak *et al.*(1968) observed the prolactin cells in the pituitaries of cows. Baker *et al.*(1969) distinguished somatotrophs and mammotrophs by the peroxidase labeled antibody technique. Everett and Baker (1945) found degranulation of LTH cells during lactation in rats. Similar observation was made by Dawson (1946) in the cat and by Desclin (1947) in the rat. Simultaneous degranulation of one group of acidophils with increase in riboproteins during pregnancy, pseudopregnancy and lactation was observed by them in rats. After castration and estrogen treatment there was degranulation in LTH acidophils (Purves and Griesbach,1952). Vanha-Peritula (1966) could identify a cell type with orangeophilic properties after PAS-orange G staining method



and they increased in number during pregnancy, lactation and after treatment with estrogen. The LTH cells are carminophilic with azocarmine-orange G method. They were scanty in male rats and absent in immature rats and are distributed singly bordering a sinusoid. They are seen throughout the whole gland in adult female rats. The nucleus is ab-sinusoid in position. Palisadic arrangement in the same location was sometimes observed specially during lactation. The same arrangement could be observed after reserpine treatment. Depending on these findings Vanha-Perttula considered these cells to be LTH cells. They stained bright red with tetrachrome method. "These cells were evenly red-stained in the pituitary gland of lactating female rats separated for 12 hours from the litter, but only diffuse red granules were present when the young were allowed to suckle for one hour. These changes are in agreement with the previous findings about the pituitary content of LTH in corresponding states."

*Ultrastructure of Mammothrophs (fig 6.3) :*

Chen *et al.* (1969) autografted pituitary glands to the kidney in rats and studied the level of LTH secretion by such glands. It was equal to the greatest amount secreted during the time of highest estrogen secretion in the estrous cycle. Infusion of extracts from the median eminence into the renal artery of rats having pituitaries autografted into the kidneys was done by Evans and Nikitovitch-Winer (1969) and Evans and Averill (1970). Increased synthesis and release of gonadotrophins and thyrotrophin were noted. This proves that reactivation of the involuted cells in the graft could happen.

*Costoff's observation on ultrastructure of the anterior pituitary autografted to the kidney capsule in female rats :*

In such circumstances increased number of mammothrophs and more active chromophobes were the primary cell types. They had increased amount of cytoplasm, vesicular endoplasmic reticulum and increased number of mitochondria. There was hypertrophy of prolactin cells with well-developed lamellar endoplasmic reticulum, hypertrophied Golgi and many granules. Other cell types were small and of lower activity. Well-developed MSH cells were noted. Parts of the graft were necrotic and some cells had plenty of lysosomes and lipofuchsin droplets.

Desclin (1950) autografted the pituitary to the kidney capsule in rats. The vagina became mucified after injection of estrogen. This is an effect of progesterone and its secretion depends on LTH. Estrogen stimulates the secretion of LTH.

For LTH secretion by the autograft estrogen stimulation is not necessary (Everett, 1954). In adult estrus rats pituitary glands were autografted to the kidney capsule and the uteri were traumatized after 4 days. Decidual tissue was present after eight days. For the formation and maintenance of the decidual tissue progesterone is required. Plenty of acidophils were encountered in the grafts (Everett, 1956) which increased in size and number with estrogen treat-



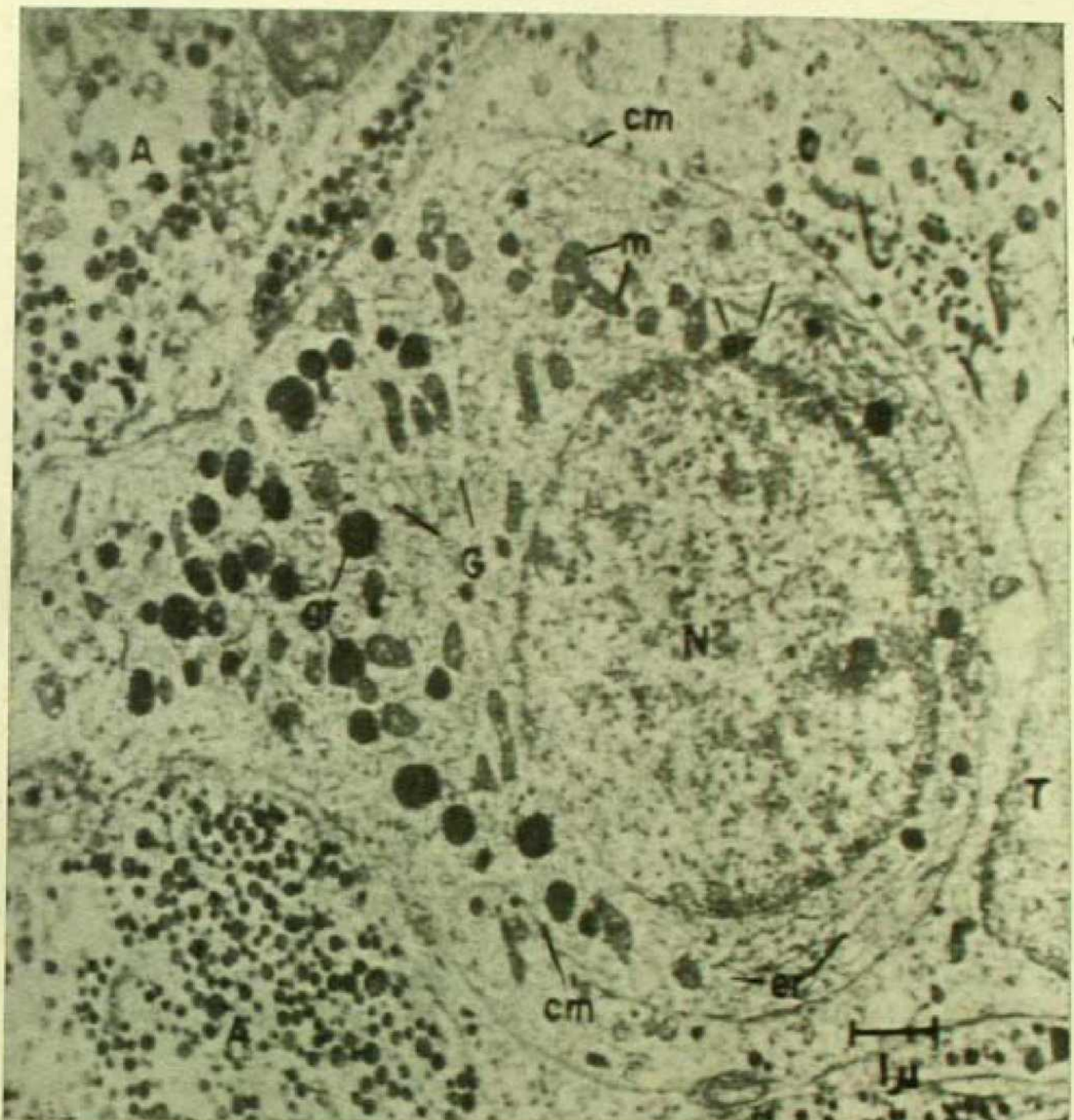


Fig. 6.3. Electron micrograph of a section from the anterior pituitary of a normal, young adult female rat showing an acidophil of the type which is thought to be responsible for the production of mammotrophic hormone (Hedinger and Farquhar, 1957; Farquhar and Rinehart, 1954a). The nucleus (N) and the cell membrane (cm) of the mammothroph are shown.

These cells are typically found alone, rather than in groups, in the normal, nonlactating animal. Their most distinctive feature in electron micrographs is their cytoplasmic content of very large, dense secretory granules (gr) with a maximal diameter of 600 to 900 m $\mu$ . In this cell they are predominantly found grouped to the left of the nucleus (N). The granules appear very dense and do not show evidence of internal structure. Their appearance is in contrast to that of the mitochondria (m) which are usually more elongated, much less dense, and show clear internal structure which is difficult to see in detail at this relatively low magnification.

Tubular and cisternal (elongated) profiles of the endoplasmic reticulum (er) as well as vacuoles of the Golgi complex (G) may also be identified in the cytoplasm. The two areas marked A represent segments of the cytoplasm of two adjacent acidophils of the type which are presumed to be responsible for



ment. Rennels (1962) observed the LTH cells in the pituitary grafts under the renal capsule. Desclin and Flament-Durant (1966) observed rapid degranulation of LTH cells in the renal pituitary grafts after estrogen treatment. Tissue culture experiments uphold the same view as observed in grafting experiments (Pasteels and Mulnard, 1961).

Costoff (1973) observed that 10% of the cell types in male rat pituitaries are mammotrophs whereas pituitaries in the females comprise 40% cell population. In the male this cell type is third most frequent cell type in order after somatotrophs and follicle-stimulating gonadotrophs. In the female this cell type is plenty like somatotrophs. The luteotrophs are found in the central part of the gland and they are associated with corticotrophs.

The LTH cells are similar to somatotrophs in shape and size but when stimulated they may be double in size with irregular shape. This cell could easily be identified because of intensely electron-dense and irregularly shaped granules. The granules measure 100m $\mu$  in diameter within the Golgi region but the mature granules were about 800m $\mu$  in diameter. Fewer granules were noted in the mammotrophs. Mitochondria are rodshaped. More lysosomes are noted in the mammotrophs than in the somatotrophs. The endoplasmic reticulum is well-developed and extensive. The Golgi apparatus is extensive in the luteotrophs in pregnant rats. Usually several Golgi complexes have been noted in LTH cells.

#### *Adrenalectomy :*

In short term experiments there were more prolactin cells with increased granulations.

#### *Castration and adrenalectomy :*

Prolactin cells were conspicuous and had dilated endoplasmic reticulum. Swollen mitochondria and increased granulations were observed in these cells.

#### *Adrenal cortical blockers :*

Number of mammotrophs decreased.

#### *Estrus :*

Degranulation was observed in some mammotrophs.

the production of growth hormone. The smaller size of the secretory granules distinguishes these cells from the mammotrophic acidophil.

A portion of the nucleus of a thyrotroph (T) is seen to the right. Cytoplasmic processes of this cell extend out from the nucleus to encircle partially the mammotroph. The cell may be identified as a thyrotroph on the basis of its angular shape and content of very small secretory granules.

In the lactating animal acidophils of this type with large secretory granules are very numerous and can be seen in virtually every field (Hedinger and Farquhar, 1957).  $\times 11,700$ . Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



The granules in the LTH cells varied in diameter from 250-870m $\mu$  with a mean of 318m $\mu$ . The prolactin activity was found in the E2LSP zone and granules contained therein had a mean diameter of 361m $\mu$ .

#### *Adrenocorticotrophs :*

Vanha-Perttula (1966) identified ACTH cells in the pituitary gland of rats after adrenalectomy when there is increased ACTH production. An increase of a special type of amphophil cell was also detected and it differed from chromophobes by its greater size. With PAS-orange G and azocarmine-orange G methods small orangeophilic granules were noted in these cells. With tetrachrome method there were erythrosinophilic granules at the luminal border of the cell and they could thus be differentiated from the chromophobes. The granules diminished from ACTH cells after cortisone treatment with decrease in the size of the cells. ACTH cell is oval when lining the sinusoid, polygonal when situated alone amidst other cell types. Sometimes a palisadic arrangement could be seen.

#### *Basophils as a source of ACTH :*

Hylinized basophils were described by Crooke and Russell (1935) in Cushing's disease.

Knigge (1955, 1956, 1957) thought that cells stained by protargol method secreted ACTH. These cells responded during stress and had relations to cells that were PAS +. Basophils decreased and the cytology was changed after adrenalectomy. He thought that ACTH was produced by basophils. Halmi and Bogdanove (1951) and Dhom *et al.* (1962) suggested that aldehyde fuchsin + basophils secrete ACTH and TSH.

Immunofluorescence studies show that some basophils secrete ACTH (Marshall, 1951, Pearse and Van Noorden, 1963, Hess *et al.*, 1968).

#### *Acidophils as the source :*

Stress responses after severe burns in the rat were studied by Finerty and Binhammer (1952) and Finerty *et al.*, (1952). By staining with azan method there were no changes in the differential cell counts and in the degree of specific granulations of the cells. When acid hematein method of Rennels (1951) was applied to the sections, an increase in the number of acidophil cells per field was found. This discrepancy was resolved by Timmer and Finerty (1956) by stating that in scalding a shrinkage in the gland takes place and the crowding of the acid hematein stained cells led to the increase in such cells per field.

However, Roy (1953/1954) observed increase in basophils in burn trauma in the man and dog.

Acidophilic granules have ACTH activity (Stigliani *et al.*, 1954) and Field (1958) noted a decrease in acidophils after adrenalectomy. Synthesis of ACTH





Fig. 6.4. Electron micrograph illustrating portions of several cells which were recently suggested to be concerned with the formation of adrenocorticotrophic hormone (Farquhar, 1957). Two large nuclei (N) and a segment of a third nucleus are shown.

Such cells are typically found in groups and are arranged around large follicles of smaller ductiles. Some of the follicles are quite large, measuring several microns across and are undoubtedly analogous to so-called "colloid cysts" sometimes seen in the anterior lobe by light microscopy. Other follicles, such as the one illustrated here, are quite small and would probably escape detection by light microscopy.

The cells which line the follicles or ductiles typically show tiny cytoplasmic projections or microvilli (mv) which project into the follicular lumina. In this field portions of three cells abut on the follicle and form microvilli which project into the lumen.

The follicular cells characteristically do not contain secretory granules, organized cytoplasmic structures are sparse. Only a few mitochondria (m). Furthermore, in the normal animal their cytoplasm appears relatively empty for occasionally tubular profiles of the endoplasmic reticulum (er), and basophilic particles (bp) are encountered.



is in the acidophils. Isolated granules (0.5-1.0 $\mu$  in diameter) from sheep pituitaries contain ACTH activity (Herlant, 1953). By immunofluorescence studies it has been suggested that ACTH is synthesized in acidophils (Leznoff *et al.*, 1962; and Kracht *et al.*, 1965).

#### *Amphophils as the source :*

Russfield (1957) classified the cells of the human pars distalis into acidophils, basophils, amphophils, hypertrophic amphophils, and chromophobes. The amphophils stain with both acidic and basic dyes and ACTH is being secreted by them (Burt and Velardo, 1954; and Russfield *et al.*, 1956). Amphophils may also be the sources of STH, TSH, LTH, and the gonadotrophins and their shape is polygonal. These cells are partially degranulated basophils with plenty of acidophilic mitochondria. They are sensitive to postmortem autolysis and hypertrophic amphophils are gamma cells with loss of cytoplasm and granules by postmortem autolysis. The studies were performed in tumorous conditions (Russfield, Reiner and Klaus, 1956) and endocrine disturbances (Burt and Velardo, 1954). Russfield thinks that amphophils can produce all the anterior lobe hormones but there is no proof that all of them are simultaneously produced. Purves (1961) states, "This seems to mean no more than that large amounts of hormone may be secreted by lightly granulated cells. Russfield's results are of importance in directing attention to the fact that more information of endocrinologic significance can be obtained from the study of lightly granulated cells than by the enumeration of *typical* acidophils and basophils; they do not conflict with the view that the cells producing different hormones are characterized by different types of granules, whose specific character can be distinguished by appropriate staining methods".

#### *Chromophobes as the source :*

ACTH was secreted by chromophobes (Purves and Griesbach, 1956). Ultrastructural studies led Farquhar (1957) to think that angular chromophobelike cells having microvilli and facing a colloid-filled follicle might be the source of ACTH (fig. 6.4). This colloid increased after cortisone treatment and decreased after adrenalectomy. Rennels (1964) partly confirmed the above findings. Herlant and coworkers found that all the cells with erythrosinophilic granules are not necessarily prolactin cells. In both sexes of the rat and man there are cells which seem to be chromophobes but with tetrachrome staining these cells

In terms of their somewhat monotonous regularity, these cells resemble more closely the cells from the intermediate lobe than they do any other type of anterior lobe cell.

Because of the response of these cells to alterations in adrenal activity and their lack of response to other experimental procedures, it has been tentatively suggested (Farquhar, 1957) that these cells are responsible for the formation and/or transport of corticotrophin  $\times 10,800$ .

These follicular cells are now thought to produce no hormone. Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



contain a seed-bed of fine erythrosinophilic granules which are usually polar in distribution. Herlant(1964) says that these cells secrete ACTH. In the rat after bilateral adrenalectomy there is massive hypertrophy of this cell type only (Quenum and Herlant,1964; and Kraicer and Herlant,1964). This is also observed after administration of amphenone or metopirone which strongly stimulates the secretion of ACTH (Racadot and Herlant,1960; Herlant and Klatsky,1963; Racadot,1963). Kraicer and Herlant(1964) observed selective involution of this cell type after prolonged administration of hydrocortisone. Purves(1966) stated, "In my opinion the gamma cells of Romeis in the human pars distalis, the epsilon cells of Herlant and Racadot(1957) in the pars anterior of the cat, and zeta cell of Goldberg and Chaikoff(1952) in the pars anterior of the dog, are corticotrophs."

In man the ACTH cell is a debated subject. Beta-cells secrete ACTH. This has been thought because of the following two facts. Hyaline transformation of the beta-cells occurs in hypercorticot states. Basophilic adenoma in the pituitary body has been noted in Cushing's syndrome with hypersecretion of the adrenal cortex. Herlant(1964) said that Crooke's cells are not the cause of hypercorticism, but rather the consequence. The cells composing the adenomas in the pituitary body contain fine erythrosinophilic granules.

Corticotrophs could be identified in cats in various pathological and physiological conditions (Racadot and Herlant,1960; Racadot,1963; and Quenum and Herlant,1964). They noticed that cells in chromophobe adenoma contained many fine granules that could be stained by tetrachrome method which is a revised tetrachrome technique over Cleveland-Wolfe trichrome method. In some chromophobes increase in granulation after adrenalectomy and metopirone treatment was observed. Fine acidophilic granules decreased after hydrocortisone. Changes in the thyroid gland and reproductive tract were noticed by Racadot(1963) in cats after administration of amphenone (adrenocortical blocking agent). Amphenone caused increased granulation in ACTH cells; but it was found later on that this compound stimulated granulation in mammotrophs which led to milk secretion in cats.

By tetrachrome method and immunofluorescence technique Brozman(1967) confirmed the findings of Herlant-group regarding the identification of ACTH cells with fine granules.

Light-microscopic autoradiography has been used for identification of ACTH cells by Siperstein(1963) and Knutson(1963, 1966a,b). Siperstein(1963) used pituitary sections from adrenalectomized rats receiving  $^3\text{H}$ -glycine. Large chromophobic cells which on the basis of grain counts appeared to have a higher turnover of protein than any other cell type. They constituted 1.3 to 1.8% of the cell population and were thought to be the source of corticotrophin.





Knutson(1966) observed an increase in number and size of chromophobes upto 8 days after adrenalectomy but there was labeling of some basophils 14 days after adrenalectomy. He thought that when demand for ACTH was high, it was secreted not only by chromophobes but also PAS positive basophils synthesized the hormone. Stress in the intact rat could increase RNA synthesis in chromophobes.

#### *Ultrastructure :*

Siperstein and Allison(1965) studied the pituitary glands seven days after adrenalectomy in rats. Corticotroph was a large and irregularly shaped cell having few granules and a vacuolated endoplasmic reticulum. The diameter of the granules was about 200m $\mu$ . Kurosumi and Kobayashi(1966) and Kurosumi and Oota(1966) observed greatest granulations in the cells under similar circumstance, four days after adrenalectomy. Degranulation and degeneration was noticed in many ACTH cells seven days after adrenalectomy. The granules were 150-200m $\mu$  in diameter. Yamada and Yamashita(1967) studied ACTH cells in mice and they were similar to such cells as previously described.

#### *Corticotrophs in rats (Costoff,1973) :*

Corticotrophs are found in the posteromedial part of the gland. They are large and irregular in shape and are smaller than FSH gonadotrophs. Narrow cytoplasmic projections from these cells between other cells. They stay in groups and are also associated with LTH and FSH cells. This cell-type has got variegated appearances regarding the granules. Some are electron dense, others are disrupted and in some there is a dense core surrounded by a halo under the granule membrane. The mean diameter was 106m $\mu$  and the range was 50-180m $\mu$ . The nucleus is eccentrically situated and irregular in shape with numerous Golgi regions which encircle the nucleus. They consist of long, flattened saccules and vesicles. The endoplasmic reticulum is vesicular. Ribosomes are seen to be attached to the endoplasmic reticulum but free ribosomes in the cytoplasm can also be seen, as also rosette-shaped figures, and centrioles with cilia are noted. Lysosomes are plenty. Mitochondria are of irregular shapes and sizes and they are clustered around the Golgi regions. The mitochondrial cristae are irregular and scanty and the matrix is clear.

After adrenalectomy ACTH cells were somewhat degranulated at one or two days, but corticotrophs with increased granulation were noted after five days. Mitotic figures were few. Costoff (1973) further said that, "degranulated somatotrophs 4 days after adrenalectomy may degenerate into chromophobes which in turn may differentiate into ACTH cells".

#### *Adrenalectomy cells*

Several days after adrenalectomy, the rough surfaced endoplasmic reticulum of adrenalectomy cells forms dilated cisternae and they are filled with colloid-like



substance. This is due to increased production of newly synthesized protein in the rough endoplasmic reticulum. The same feature is found also in thyroidectomy cells and castration cells. In some cells the cisternae are dilated and in some they are flat. Whorl-like or concentrically arranged membranes of the endoplasmic reticulum have been noted in some adrenalectomy cells by Kurosumi and Kobayashi(1966). They are smooth-surfaced which is due to detachment of ribosomes. These ribosomes are found in clusters in the cytoplasm near the whorls. They may be evidences of degeneration.

Gosbee and Kraicer(1970) studied rat pituitary glands 1 month after adrenalectomy by autoradiography. Increase in the label in the cells of the pars distalis did not occur, instead the increase in the label was in the cells of the pars intermedia. They suggested that in this situation cells of the pars intermedia were active and may be related to corticotrophin synthesis and release. Costoff(1973) concludes, "the evidence for the increase in pituitary ACTH after longterm adrenalectomy cannot be wholly accounted for by the amount of hormone associated with granules present in the cell identified as a corticotroph. It appears that the hormone is released as soon as it is formed, is stored in a follicle prior to release, or perhaps MSH cells or another type of cells of the pars intermedia produce an ACTH-like hormone".

Costoff(1973) studied the effect of adrenal cortical blockers, adrenal steroids, ACTH and  $\alpha$ -ethyltryptamine on corticotrophs.

Racadot and Herlant(1960) and Racadot(1963) studied pituitaries of cats treated with amphenone and Metopirone by light microscopy. There was hypertrophy of corticotrophs with increased granulation. Stimulation of mammothrops after amphenone treatment was also noted. Hypertrophy of corticotrophs in Gambian rats treated with Metopirone was noted by Quenum (1964). Costoff (1973) did not find increase in size or granulation of ACTH cells. There were different degrees of degranulation. After Metopirone treatment hypertrophy of the cisternae of the endoplasmic reticulum was observed. This variation in the observation is due to small physiologic doses of the drugs used. After amphenone treatment nuclear inclusions, greatly invaginated nuclei and swollen mitochondria in corticotrophs were found. Costoff thinks that these compounds act directly on the pituitary and the adrenal cortex. This confirms Steenburg's (1965) postulation.

#### *Dexamethasone treatment :*

There was inhibition of corticotrophs. The small number of corticotrophs present were smaller in size and found to be degenerating. The Golgi complex was found to be atrophic. Fragmentation of the endoplasmic reticulum with few ribosomes was also observed. Swollen mitochondria with loss of cristae were



evident. The cytoplasm was vacuolated and degranulated with increase of lysosomes.

*Hydrocortisone treatment :*

Treatment for *five to seven days* showed that part of the corticotrophs was well granulated. The Golgi complex was inactive. The cisternae of the endoplasmic reticulum were dilated in some cells and in others there were poorly organized fragments. Free ribosomes were noted in the cytoplasm and this indicates a diminishing function. Short rod-shaped or round mitochondria were found. Lysosomes were few in number. There was no change in other cell types.

*14 days of treatment :*

Crooke's changes were noted in the pituitary and there was vacuolation in the cytoplasm. No corticotrophs were observed. Other cell types could not be discerned as the cell membranes could not be distinguished.

*30 days of treatment :*

Lipid droplets and vacuolated cytoplasm were the pictures. Round and swollen mitochondria were found. Golgi complex was absent from many cells. Connective tissue infiltrated around capillaries.

*ACTH treatment :*

Greatly stimulated corticotrophs were observed. "It probably acted on the adrenal cortex which allowed the corticotrophs of the pituitary to store their hormone in granules rather than secrete it".

*$\alpha$ -ethyltryptamine treatment :*

It prevented the release "but apparently not the synthesis of ACTH since these cells were well granulated".

*Adrenalectomy + castration :*

The corticotrophs appeared to be the same as noted after adrenalectomy alone. Swollen mitochondria with broken cristae and increased granulation were noted. Large and irregularly shaped corticotrophs were not observed as is noted in adrenalectomized rats. Castration and lack of estrogen "prevent release of high level of CRF and more corticotrophin would be stored than released".



*Gonadotrophs (figs. 6.5 and 6.6) :*

*After Vanha-Perttula (1965) (rat pituitary gland)*

Hormone	Staining (Mallory)	Cell type (Herlant)	Special feature	Reference
LH or ICSH	basophil	Gamma	PAS: +, central round PAS-red, peripheral  Immunofluorescence (man)	Purves & Griesbach, 1954, 1955, 1956 Rennels, 1957, 1963 Hildebrand <i>et al.</i> , 1957 Midgley, 1963; Koffler & Fogel, 1964; Robyn <i>et al.</i> , 1964
FSH	basophil	Beta	PAS: +, peripheral  PAS-purple, central  Immunofluorescence (man) (pig)	Purves & Griesbach, 1954, 1955, 1956 Rennels, 1957, 1963 Hildebrand <i>et al.</i> , 1957 Koffler and Fogel, 1964 Della Corte & Biondi, 1964

FSH was thought to be secreted by peripheral, coarse granulated PAS-positive cells and LH by centrally situated, larger and fine granulated PAS-positive cells (Purves and Griesbach, 1954, 1955). Reverse location of these cells in the rat pituitary gland has been noted by Rennels (1957, 1963), Hildebrand *et al.* (1957), and Hellbaum *et al.* (1961). Some cells stained red with PAS-methyl blue-orange G method and are situated peripherally in the *sex-zone*. With increase in LH activity after gonadectomy there is an increase of this cell type. When PAS-purple central gonadotrophs increased in number, there was heightened FSH activity. Rennels (1963) said that differences in strain could explain a part of this controversy.

Vanha-Perttula (1966) observed similar staining for FSH and LH cells. They could be differentiated by the alcian blue method at pH 0.2 and the aldehyde-thionin method. With the alcian blue method FSH cells are stained violet because they have affinity for alcian blue and PAS, while aldehyde-thionin stains only LH-cells. With azocarmine-orange G method FSH-cells are violet to certain extent due to small granules. FSH cells were distributed peripherally in the ventral part of the gland but a large group of cells were noted in the so called



*sex zone* adjacent to the intermediate lobe. FSH-cells are round or oval, PAS-positive and are grouped around the sinusoids. There are coarse granules in them but they leave a round Golgi zone free. Enlargement of these cells occur after castration and vacuoles appear in the cytoplasm after a longer time. Signet ring-cells appear thirty days after castration. A large number of FSH-cells was noted on the ventro-caudal border of the pituitary gland in young rats of about 30 days of age.

In the normal male and female rats LH or ICSH-cells are few in number. After castration and during pregnancy the number of PAS positive-cells is increased to a great extent. At pH 0.2 these cells have only weak affinity for alcian blue and remain red due to PAS. At pH 3.0 these cells are coloured, light-green and centrally situated but can be easily differentiated from the TSH-cells by their round or oval shape and lighter staining affinity in methods like tetrachrome, acid fuchsin-aniline blue and azocarmine-orange G. The glycoprotein granules are finer than those of FSH cells. These cells are intimately connected to the sinusoids without forming larger groups.

Gonadotrophic and thyrotrophic cells have a mucoid, PAS-positive component having affinity for aldehyde-fuchsin and alcian blue. Differences in the staining reaction to those dyes speak in favour of different chemically reactive groups. By combined performic acid-alcian blue-PAS-orange G technique, Adams and Sweetenham(1958) could differentiate two cell types in the human hypophysis. The S-cells are responsible for ACTH synthesis in man. They are rich in cystine. Red stained R-Cells producing FSH contain much carbohydrate (Adams and Sweetenham,1958; Adams and Pearse,1959; and Sweetenham,1960). Dhom and Tietze(1962) recommended identical staining method in the rat for differentiation of FSH and ACTH cells. Vanha-Perttula (1966) could not confirm this suggestion in the rat, "it is apparent that the staining with PAS and aldehyde-fuchsin as well as aldehyde-thionin in gonadotrophic and thyrotrophic cells is due to different material. The contribution of the sialic acid component of FSH (Racadot,1963; Rennels and Hood,1964; and Rennels, 1965) to these reactions also remains to be solved".

FSH and LH cells can be distinguished by autoradiography and immunofluorescence method.

#### *Autoradiography :*

Tritiated proline was found to be incorporated into gonadotrophs of castrate animals by Ducommun(1965). After thyroidectomy more tritiated proline was incorporated into TSH cells. Thus gonadotrophs could be distinguished from thyrotrophs.  $^3\text{H}$ -leucine was incorporated into the gonadotrophs of castrate and estrogen treated rats (Kobayashi *et al.*,1967).



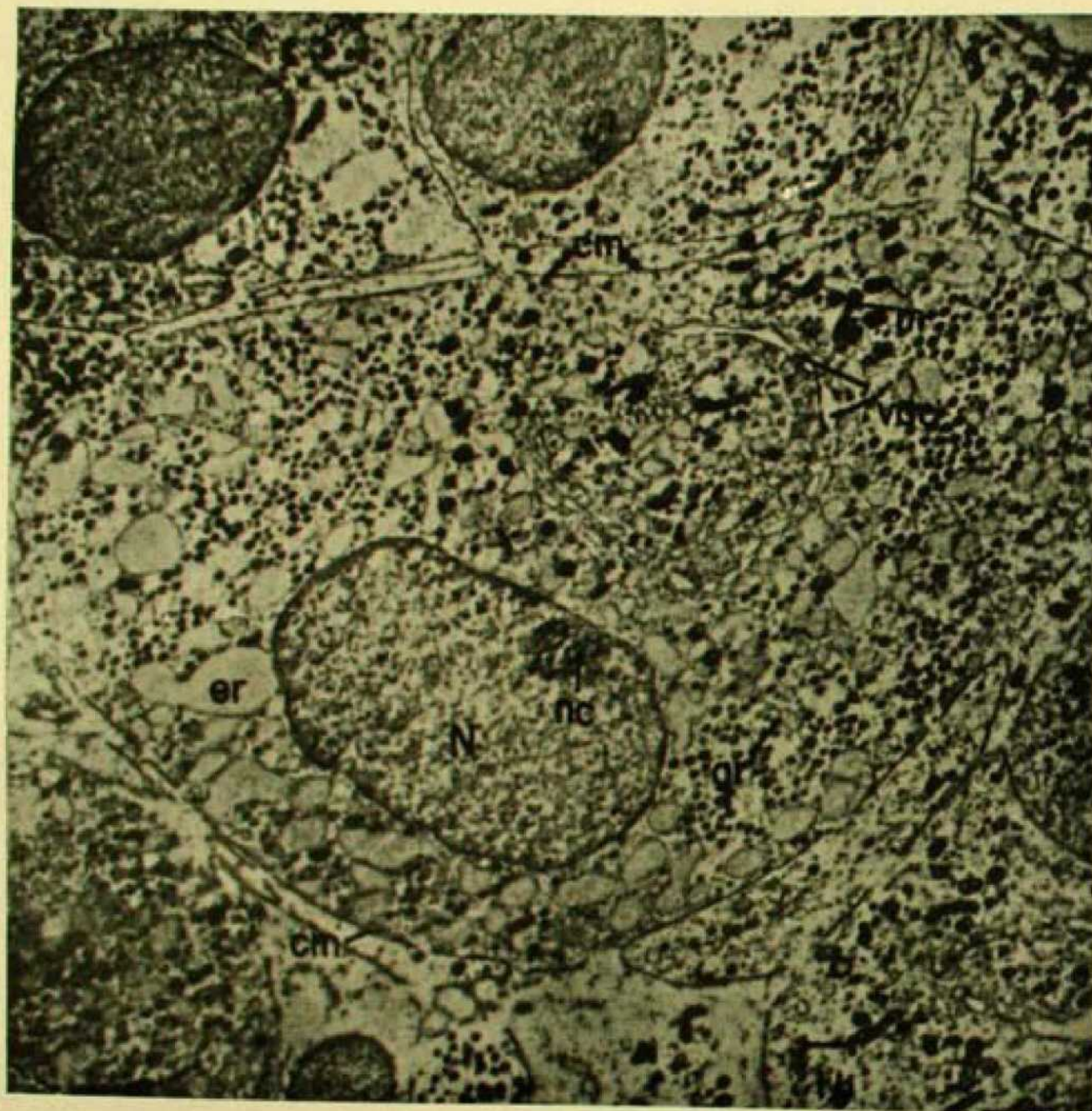


Fig. 6.5. Electron micrograph showing a very large gonadotroph from the anterior pituitary of a young adult male rat. The nucleus (N), a nucleolus (nc), and the cell membrane (cm) are shown.

This cell can be identified as a gonadotroph by virtue of its rounded contours and content of secretory granules (gr) with a maximal diameter of approximately 150 m $\mu$ . The secretory granules of gonadotrophs are intermediate in size between the large secretory granules of acidophils and the small secretory granules of thyrotrophs.

A spherical chain of small vacuoles (vac) circumscribes the Golgi apparatus which is located above the nucleus. Elements of the Golgi complex outline a cytoplasmic area nearly as large as the nucleus.

Mitochondria (m) which have been sectioned in various planes are also visible in the cytoplasm. The mitochondria of gonadotrophs are generally more elongated and show a denser internal background matrix than other types of adenohypophysial cells.

The endoplasmic reticulum (er) is seen here in the form of numerous vesicles which vary greatly in size. Some are relatively small and are of size approaching that of the secretory granules. Others are rather large, for they measure several microns across at their greatest width. It can be seen that the internum of the



*Immunofluorescent technique;*

Man	— LH cells	— Midgley (1963), Robyn <i>et al.</i> (1964), Bain and Ezrin (1970).
Man	— FSH and LH cells	— Koffler and Fogel (1964) Fogel and Koffler (1964)
Pig	— FSH cells	— Della Corte and Biondi (1964)
Pig	— LH cells	— Pomerantz and Simmons (1968)
&		Herlant and Ectors (1970)
Cow	•	
Rat	— LH cells	— Monroe <i>et al.</i> (1969)

Rennels(1963) used antisera to ovine LH and FSH with the indirect immunofluorescence procedure for demonstrating two types of cells in ovine hypophyses which fluoresce in contrasting colours. There are indications that LH and FSH cells are of distinct entities.

Nakane(1970) used peroxidase-labeled antibody procedure and found that some gonadotrophic cells have both FSH and LH and thus he invalidates the one-cell-one-hormone hypothesis. This view has been supported by the finding of Costoff and McShan (1969) that in the most pure fractions of pituitary secretory granules FSH and LH activities are associated with granules of the same size (average 150 m $\mu$ ) and density.

Barnes(1963) studied the fine structure of the mouse adenohypophysis in various physiological states.

*FSH cells:* These cells are located adjacent to blood vessels and are large rounded or oval. The granules vary from 150—200m $\mu$  in size. The endoplasmic reticulum appears as a series of dilated sacs. These cells normally occur in two forms.

*Form I:* The endoplasmic reticulum is not prominent. They are roughly circular but small. The mitochondria are rod-shaped with an electron-dense matrix.

*Form II:* Hypertrophied form of FSH cell: The endoplasmic reticulum is very prominent. They occur as a series of grossly dilated sacs of irregular outline. "Lakes of relatively electron transparent amorphous material, deposits of

vesicles appears homogeneously grey, and has a background density greater than that of the surrounding cytoplasmic matrix.

Gonadotrophs with this appearance have been associated with the secretion of follicle-stimulating hormone (Farquhar and Rinehart, 1954a; Farquhar and Rinehart, 1955). They differ from the luteinizing hormone-gonadotroph (see Fig. 6.6) in possessing somewhat paler nuclei, more evenly distributed granules, and prominent vesicles of the endoplasmic reticulum with the homogeneous grey internum. X 6500.

Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



denser granular material and membrane bound vacuoles are commonly encountered in this form of FSH cells". Mitochondria are rod-shaped.

*LH cells:* These are small, rounded or polygonal in shape and usually on or close to blood vessels. The secretory granules are electron—dense and ranges from 100—200m $\mu$ ; but smaller (75m $\mu$ ) and larger (300m $\mu$ ) granules are not uncommon. The ratio of cytoplasmic to nuclear volume is smaller than in other types of pituitary cells. They occur normally in two forms.

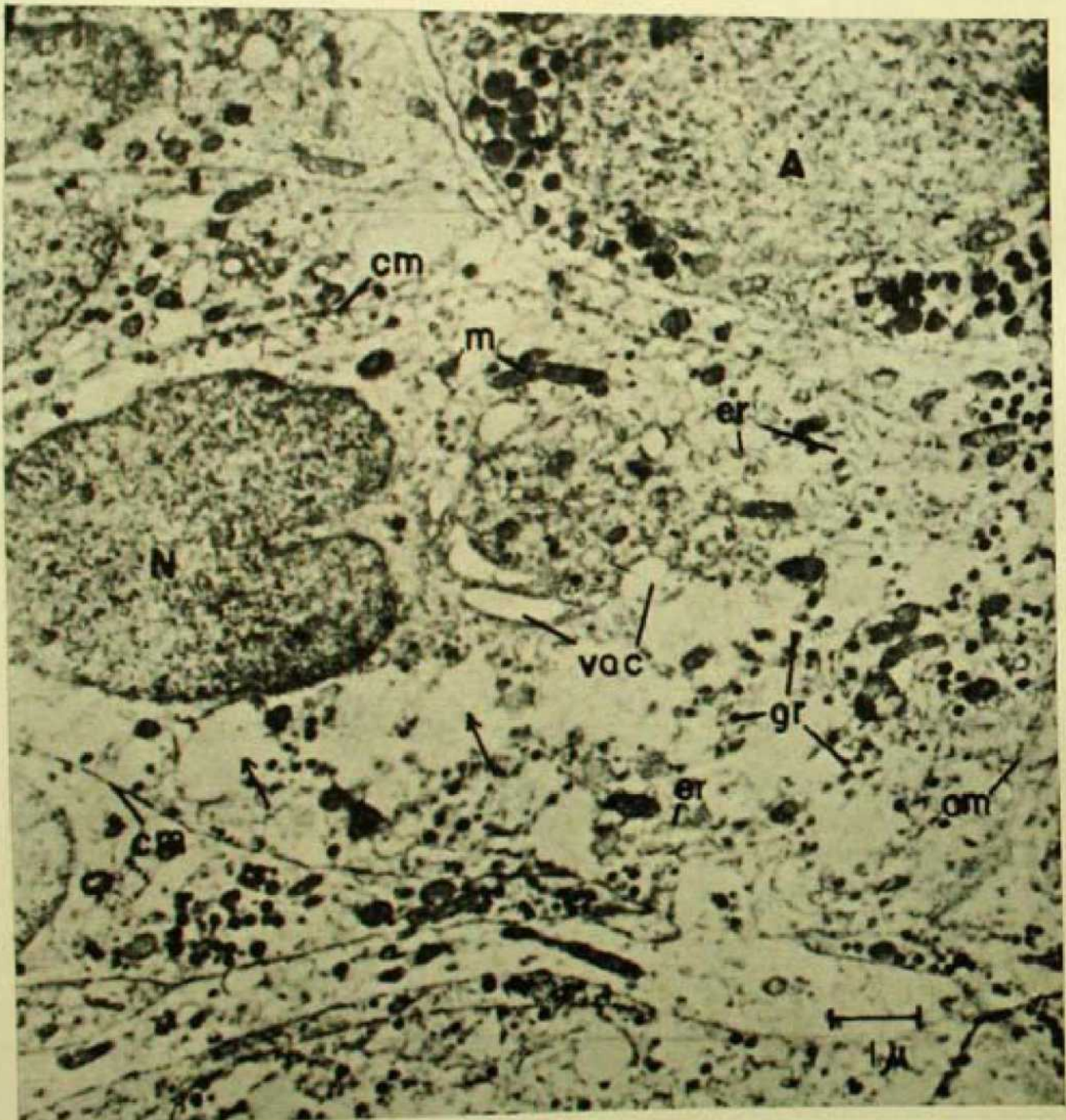


Fig. 6.6 Electron micrograph illustrating another gonadotroph from the anterior pituitary of a young adult male rat. Like the cell in Figure 6.5, this cell can be identified as a gonadotroph on the basis of its rounded contours, the size of its secretory granules (maximal diameter ca. 150 m $\mu$ ), and elongated mitochondria (m) with a dense internal matrix. The area occupied by the Golgi complex is seen directly to the right of the nucleus. It is outlined by a number of relatively empty-appearing vacuoles (vac).



*Form I*: The Golgi apparatus is small and poorly developed. The endoplasmic reticulum is scattered, flattened and oriented parallel to the outline of the nucleus.  
*Form II*: The endoplasmic reticulum and Golgi apparatus are more prominent and the cisternae of the endoplasmic reticulum are grossly enlarged.

"No electron-dense material has been observed within the cisternae of the endoplasmic reticulum in either form of LH cells".

She also studied the female mouse from sexual maturity to senility. Poorly granulated FSH and LH gonadotrophs were found in the pituitary of the diestrous females. Little development of cytoplasmic membrane systems could be noticed. There is dramatic change in the pituitary as the animal passes from diestrous to proestrous. The FSH cells fill up first with secretory granules followed by the filling up of LH cells. Extensive endoplasmic reticulum and Golgi membrane systems in FSH cells were found. In this phase of FSH secretory cycle cytoplasmic inclusions of varying density and vacuoles have been noted. The vacuoles do not contain nucleoproteinaceous material. At this period the LH cells are well granulated but no comparable development of the cytoplasmic membrane systems is there. "By estrus both the degree of granulation and the degree of cytoplasmic membrane system development has begun to decrease in the FSH gonadotrophs whereas the LH gonadotrophs now display an arresting and often bizarre development of their cytoplasmic membrane systems. This membrane development coincides with a marked reduction in the number of LH granules present within the cell. The involution of the FSH gonadotrophs which begins around estrus, continues throughout metestrus." In the typical metestrus FSH gonadotroph there are some granules but the cytoplasmic membrane system approaches that of the diestrous FSH cell. The LH cells refill with granules. At this stage considerable development of the cytoplasmic membrane systems in many LH cells takes place. In diestrus "both the FSH and LH gonadotrophs have reverted to the poorly granulated form in which cytoplasmic membrane development and secretory granulation are minimal".

*The castrated mouse (Barnes, 1963)*:

Dramatic reaction to castration in the FSH cell occurs in both sexes by an elaboration of its cytoplasmic membrane system and by an actual enlargement in

This gonadotroph differs from the follicle-stimulating hormone gonadotroph illustrated in Figure 6.5 in several respects: the nucleus (N) is more dense and shows a deep infolding, the secretory granules (gr) are aggregated into clumps, and no large vesicles of the endoplasmic reticulum are present. In addition, there are a number of relatively open areas visible in the cytoplasm (arrows) which are occupied only by a sparse, flocculent precipitate. The endoplasmic reticulum (er) is seen here in the form of tiny tubular profiles. Gonadotrophs with these features have been associated with the secretion of luteinizing hormone or interstitial cell-stimulating hormone (Farquhar and Rinehart, 1954a, Farquhar and Rinehart, 1955).

A portion of an acidophil (A) with larger secretory granules is present above the gonadotroph.  $\times 11,700$ .

Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



cellular volume. The reaction of the LH gonadotroph to castration is similar but less intense and slow to develop. The FSH and LH cells show the hypertrophic Form II as are found in normal mature animals. Their number increases after castration and this may be due to differentiation of new cells or increased volume of pre-existing cells.

5 weeks after castration FSH gonadotroph shows increased granulation. Intense ramification of the endoplasmic reticulum throughout the cytoplasm occurs and the dilated cisternae show densely studded RNP particles on their exterior surfaces. In the cytoplasm there are lakes of relatively electron transparent amorphous material, dense granular material and lipid inclusions. The mitochondria possess fine electron dense granulation associated with the mitochondrial cristae. In the LH gonadotroph increased number of secretory granules are found with increased development of the endoplasmic reticulum and the Golgi apparatus. "The presence of numbers of either amorphous or granular cytoplasmic inclusions of varying electron density is characteristic of FSH castration cells, while LH castration cells rarely possess them. The presence of endoplasmic reticulum profiles of irregular shape, heavily encrusted with RNP particles is characteristic of the FSH castration cell; the presence of relatively smooth surfaced, small, circular endoplasmic reticular profiles is characteristic of the LH castration cell."

Farquhar and Rinehart(1954) studied the pituitary glands of female rats at different periods after ovariectomy. Two types of basophils responding differently to castration could be identified. One type had an increase in mitochondria and granules initially followed by vacuolation with loss of granules. 75 days after castration *signet ring* cells appeared having a peripheral rim of cytoplasm surrounding one or more large central vesicles. This type of cell is responsible for FSH secretion. Different type of change was noted in the other basophil cell type which is responsible for LH secretion. This cell type shows *filigree* appearance of the cytoplasm with vacuolation.

Girod (1966) summarised electron microscopic observation of the pituitary gland of the monkey and other species.

Gonadotrophic cells were studied by Roos (1958) throughout the estrous cycle in the rat. At the height of diestrus FSH cells were degranulated when there is wave of follicular growth taking place. FSH cells degranulated during the afternoon of proestrus. LH cells degranulated during the morning of proestrus and a peak was achieved at 4.00P.M. which is the critical period of proestrus. The second surge of FSH occurs during the critical period at the same time as LH. The LH cells are centrally located.

Kurosumi and Oota(1968) identified LH and FSH cells. They produced persistent estrus and diestrus in rats by giving estrone at birth for five and thirty days respectively. Well developed Graafian follicles without corpora lutea in the ovaries of persistent estrus rats after 5 months were detected. In these





animals the pituitary had well granulated LH cells with sparse granulation in the FSH cells. Suppression of the release of LH granules in persistent estrus happened. Atrophic FSH cells were noted in the persistent diestrus rat and the LH cells looked like those in the controls. In the ovaries of these animals there were follicles with plenty of well developed interstitial tissue.

Yoshimura and Harumiya (1965) noted the FSH granules to be 150 to 200m $\mu$  in diameter and LH granules were 200-250m $\mu$  in diameter.

*Costoff's study (1973) :*

*FSH gonadotroph :* This is the largest cell found in the male rat pituitary gland. In the male they are 30%, in the female only 10%. The FSH cells are round and located on a capillary. The nucleus is either round or indented. The granule diameter is 126m $\mu$  on an average. Large *amorphous bodies* are scattered throughout the cells. The diameter of these light-staining bodies varies from 0.7 to 1.2 $\mu$  in diameter which are formed in the Golgi complex. They have been noted also by Farquhar and Rinehart (1954), Cardell (1961) and Kurosumi and Oota (1968).

Hypertrophic Golgi areas encircle the nucleus. The mitochondria are filamentous rods of different shapes. Cristae are continuous and parallel. In hypertrophic FSH cells the endoplasmic reticulum is more irregular with dilated sacs. Lysosomes, multivesicular bodies, centrioles and sometimes a cilium can also be seen.

*LH gonadotroph :* 20% of the female rats and 5 to 10% of the males possess these cells, which are situated anteromedially in the pituitary. The cells are located on a capillary and are larger than thyrotrophs. They are polygonal in shape with eccentrically placed nucleus. The diameter of the granule is 145m $\mu$  on an average. Large amorphous body could be detected in this cell type. In the inactive state the Golgi apparatus and endoplasmic reticulum are inconspicuous. In actively stimulated cells Golgi areas are extensive and the granules are in the different stages of formation. The membranes of the endoplasmic reticulum give attachment to the ribosomes but many remain free in the cytoplasm. Mitochondria are short, rodlike or rounded. There are few lysosomes, cilia and centrioles.

*Changes in estrous cycle :*

*FSH gonadotrophs :* At middiestrus these cells are small, poorly granulated and have poorly developed endoplasmic reticulum with few ribosomes attached to them. At proestrus granules are more in number, rough endoplasmic reticulum is present and Golgi complexes are active. At midproestrus these cells are filled with granules and large bodies. Endoplasmic reticulum and Golgi complexes are well developed. During late proestrus and early estrus





degranulation of the cells took place and they appeared vacuolated and chromophobelike. During metestrus FSH cells were degranulated and in early diestrus granulations were again noted.

*LH gonadotrophs*: At diestrus these cells are well granulated and the endoplasmic reticulum and the Golgi complexes are active. During midproestrus LH cells start degranulating and at the critical period, estrus there is full degranulation. At metestrus the endoplasmic reticulum and Golgi complexes are again active and during diestrus there were plenty of granulations. Costoff (1973) remarks "It would appear, therefore, that both gonadotrophs degranulate during the critical period."

*Gonadotrophs after castration in rats*:

FSH cells did not suffer much change. LH cells began to hypertrophy one week after castration and fusion and hypertrophy of vesicles or vacuoles of the endoplasmic reticulum took place after 14 days. After one month of castration, transformation of majority of LH cells into *signet ring* castration cells was observed. Large vacuoles or lakes were filled with PAS-positive colloid-like material containing LH.

*Light-staining amorphous bodies*:

These bodies are frequently found only in the FSH cells but not always. If they are not met with then at their locations large vacuoles in the endoplasmic reticulum could be found. They looked darker after glutaraldehyde fixation. Light bodies were not noted in the pituitary glands of castrate rats but more dense bodies having the characters of lysosomes were found instead. It is possible that these lighter bodies may fuse with other vesicles to be transformed into lysosomes. Cardell (1961) noted similar large bodies in the salamander pituitary. These bodies disappeared at the breeding season when degranulation of gonadotrophs occurred. Cardell thought that these bodies might represent secretory granules or they are lysosomes. Herant (1965) considered that these bodies are glycoproteinaceous and PAS-positive. Nakayama *et al.* (1970) noted these bodies in the Golgi zone. They thought that the bodies are formed either in the Golgi apparatus or by the fusion of small secretory granules. Hormones may be stored in these bodies. Costoff (1973) states that the large granule fraction does not show any gonadotrophic hormone activity and "it appears unlikely that these organelles are secretory granules." These bodies may be pre-FSH which are transformed into FSH, comparable to procorticotrophin being transformed into corticotrophin.

*Chromophobes*

Pituitary tumors may be acidophilic, basophilic or chromophobic. Signs of hypopituitarism are present in patients with chromophobic adenomas. They may be nonsecretory with wide destruction of surrounding structures including the pituitary tissue by pressure effect.



Chromophobic adenomas are quite common in old ages of animals of certain species and strains.

Experimental production of pituitary tumors has been discussed by Furth(1955). These tumors are mostly chromophobic. Chromophobic adenomas have been studied by Schelin(1962) in man and by Holmes and Mandl(1961) in the rat. The cell contains fine granules and may be a corticotroph.

Rinehart and Farquhar(1953) said that many cells which were chromophobic to light microscopy were actually poorly granulated cells.

Severinghaus(1932, 1933) stated that in the rat the Golgi apparatus of the acidophil cell class has a different form when compared to that of the basophil cell class. It forms a net-like cap over one pole of the nucleus in the acidophil cells. In the basophil cells it is a spheroidal body situated in the cytoplasm at a distance from the nucleus. Foster(1947) confirmed these findings by Sudan black staining method. In the chromophobic cell class some Golgi bodies take up the characters of granulated acidophils while others take up the characteristics of granulated basophils. This proves that chromophobic cells are not undifferentiated "rather they consist in part at least of temporarily nonfunctioning but specifically differentiated cells" (Purves, 1961).

A relationship between chromophobes and chromophils was established by Severinghaus(1939). Embryonic parenchymal stem cells differentiate into acidophilic and basophilic chromophobes in the embryonic gland. These differentiated chromophobes then convert into respective chromophilic types. On degranulation, these chromophilic cells go back to their chromophobic state, only next to differentiate into respective chromophilic types.

Wolfe and Brown(1942) found that in rats treated with estrogen there was enlargement of the Golgi body of acidophil cells and they became more or less spheroidal in shape and thus these cells could not be differentiated from the basophils by looking only at the Golgi body. This change of the Golgi body from a paranuclear net to a rounded body is also met with in castration studies when the acidophil cells diminish in number and size.

Deminatti(1959) noted that increase in the secretory granules was associated with a decrease of cytoplasmic RNA and suggested that increased protein synthesis may take place in chromophobes and a storage phase is characterized by presence of granules.

Severinghaus(1937) clarified the position of amphophils i.e. cells which have acidophilic and basophilic staining properties, in relation to his earlier observations of 1933. Highly active basophils may be mistaken for acidophils as with increased activity the basophilic granules diminished. Mitochondria of these basophilic cells are stained with acid dyes and they now may be considered as amphophilic cells. But such cells are found in pathologically affected pituitary



glands. Differentiation of acidophils and basophils from chromophobes is a reality.

At the ultrastructural level Fernandez-Moran and Luft(1949) described the chromophobic cells. These cells had a vesicular nucleus and the cytoplasm was rather clear with a few mitochondria.

Costoff(1973) classified the chromophobes into small, medium, stellate and follicle types.

#### *Small chromophobes :*

The cytoplasm of these cells was little and surrounded the nucleus. Costoff(1973) said that these cells were smaller than thyrotrophs without any granule. A few rough endoplasmic reticular vesicles, some free ribosomes, and a few mitochondria with incomplete cristae are present. The Golgi area is absent. These cells may be inactive stem cells.

After adrenalectomy the small chromophobes decreased in number while the large chromophobes increased (Siperstein,1963). The large chromophobes were active cells incorporating more labeled amino acid than other cells. These are adrenalectomy cells. Larger chromophobes grow from smaller chromophils with fine granules (ACTH cells). Thus the small chromophobes change into chromophils. Severinghaus(1937), Ezrin *et al.*(1959) and others supported this conversion. Differentiation of isolated chromophobes into acidophils and basophils happened when transplanted into the hypophysiotrophic area of the hypothalamus of hypophysectomized rats (Yoshimura *et al.*,1969). Differentiation of chromophobes into chromophils also happened in foetal rabbit pituitary glands (Schechter, 1971).

#### *Medium chromophobes :*

These cells have been noted by Costoff after castration or adrenalectomy and they differentiate into gonadotrophs and corticotrophs. The cytoplasm is two to three times more than noted in the small chromophobes. A Golgi complex (inactive) and many free ribosomes are found in the cytoplasm. Few lysosomes are present but there are no granules. Costoff(1973) thought that the pseudochromophobes were very active cells or degranulated chromophils.

#### *Follicle cells :*

Ultrastructurally these cells were described by Farquhar(1957). After hemiadenectomy the size of the follicle and the amount of colloid increased. These cells may produce ACTH. But Farquhar *et al.*(1975) stated that corticotrophs and follicular cells are distinct entities. Barnes(1961) and Kagayama (1965) described these cells in the mouse and dog respectively. The cytoplasmic processes of the stellate follicular cells in dogs contact with the perivascular spaces and they are thought to have a sustentacular function. The granules are of 200 m $\mu$ .



in diameter. These cells appear in groups surrounding colloid-filled follicle in rats (Costoff, 1973). The chromophobic cells have microvilli and cilia and desmosomes or adhesive contact areas between follicle cells. Costoff called these as pseudofollicles because there is no basement membrane surrounding the follicular wall. The cilia are of 9+2 fibre pattern. Costoff could not attach exact function to these cells but possibly they may be involved in ACTH synthesis.

In different experimental situations, Vila-Porcile (1972) and Dingemans and Feltkamp (1972) found morphological changes in follicular cells of the rat and mouse respectively. The latter authors suggested that the response is in relation to the incorporation and digestion of waste product. These follicular cells have a phagocytic function.

Farquhar *et al.* (1975) conducted *in vitro* studies on follicular cells and studied their role in phagocytosis of cells and cell debris. The phagocytic function of the follicular cells was previously reported by Farquhar (1971). They said that the long arms of the follicular cell normally extend between and surround the secretory elements, now encircle the damaged or dead cell. The arm collapses on it and subsequently puts it into the large phagocytic vacuole in the follicular cell cytoplasm. The process is similar to that noted in the octopus pulling its prey by the tentacles or that noted in an amoeba engulfing its food by pseudopodia. Acid phosphatase tests indicate that the phagocytic vacuoles have lysosomal enzymes which are capable of digesting the phagocytosed material. The energy requirement is satisfied anaerobically by their glycogen reserves or the presence of glucose in the medium. Dingemans and Feltkamp (1972) found phagocytosis in follicular or nongranulated cells of the mouse pituitaries after radiothyroidectomy, castration, adrenalectomy, and transplantation of the pituitary to the kidney capsule. Extracellularly discharged lysosomal residues may be picked up and disposed of by follicular cells (Farquhar *et al.*, 1975). So the follicular system acts as a scavenger system. There may be additional functions as yet unknown of these unusual cells.

The cell coat composed of sialic acid groups is thicker on the luminal than on the remainder of the follicular cell surface.

### Stellate cells

Vila-Porcile (1972) extensively reviewed the folliculo-stellate cell and studied the cells and the follicles in the pars distalis of the rat. They have also been studied in details in different vertebrates by Holmes and Ball (1974).

These cells have three to four cytoplasmic projections in the rat (Costoff, 1973). The size of the cell is medium. Scattered vesicles of endoplasmic reticulum, few ribosomes, and hypertrophied Golgi complex have been noted. These are agranular cells and lysosomes are rare. Rinehart and Farquhar (1953, 1955) identified these cells in the rat, Barnes (1962) in the mouse, Salazar (1963) in the rabbit, Cardell (1964) in the salamander, Forbes (1972) in the reptile *Anolis*.





Della Corte *et al.*(1968) in the lizard (*Lacerta scula*), Cardell(1964, 1969), Masur(1969), Bunt(1969), Compher and Dent(1970), Hopkins(1970), Nakai and Gorbman(1969), Doerr-Schott and Follenius(1970) in pars distalis and pars intermedia of amphibians, Knowles and Vollrath(1966) in *Anguilla*, Vollrath(1966) in the elver pars distalis, Leatherland(1969, 1970), Abraham(1971), Hopkins (1969), Weiss(1965) and Follenius(1968) in teleosts, Mellinger(1969), Alluchon—Gerard(1971) in elasmobranchs, Fernholm and Olsson(1969) in myxinoidea, and Harrison and Young(1969) in the dolphin.

Cardell(1969) found these cells throughout the pituitary having contact with all secretory cells in the pars distalis and having a sustentacular function in salamander.

Schechter(1969) considered the stellate cells to have a supportive function structurally or metabolically. After administration of metopirone, hypertrophy of only the organelles of stellate cells was found. No granules could be detected in these cells. They have a supportive function. Subsequently, Schechter(1971) thought that the corticotrophs may arise from stellate cells.

Holmes and Ball (1974) studied the importance of the stellate cells. These cells have been found in most classes of vertebrates. They have been noted in *Myxine* and in the proximal pars distalis of lampreys. In elasmobranchs the pericavity cells which line the spaces of the adenohypophysis are probably part of the folliculo-stellate cell system. These spaces are equivalent to the hypophysial cleft. In the teleost pars distalis the neck cells and the interstitial cells may be equivalent to stellate cells. They may form pseudofollicles containing extracellular colloid, as is found in the rat, or the processes of the stellate cells proceed between the secretory cells. In *Gasterosteus* the cell bodies are peripherally situated in the gland.

Typical stellate cells have been described in amphibia and reptiles in relevant chapters.

The cells have close relation to blood vessels or to perivascular channels by endfeet of protoplasmic processes or by a direct cytoplasmic contact with basement membranes. They are also closely related to the granular endocrine cells. The stellate cells may take part in intraglandular transport system or in the process of regulation of metabolism and circulation of the interstitial material (Vila-Porcile, 1972).

Cosoff(1973) thought the stem cells to differentiate into chromophils and medium chromophobes to differentiate into granulated cell types. Stellate chromophobes have a sustentacular role. Certain hormones may be synthesized in the follicular cells.



### *Pars tuberalis*

The *pars tuberalis* (*Pars infundibularis adenohipophyseos* of German authors) develops from the lateral lobes and is in close contact with the median eminence usually covering the same. The *pars distalis* probably always includes the proximal parts of the lateral lobes. Wingstrand(1966) said, "In some birds and some reptiles these basal parts of the lateral lobes can be seen in the adult gland as a band of cells, different from those of the *pars distalis* proper. This part was called *pars tuberalis interna* (Wingstrand, 1951) to avoid confusion with the *zona tuberalis*, which has a different and seemingly variable embryonic origin and is dependent on the action of the portal blood. In monotremes, birds, and many reptiles, the following part of the lateral lobes bridge the gap between the adenohipophysis and the median eminence and usually fuse to an unpaired string around the portal vessels. This string was called the *porto-tuberal tract* by Benoit and Assenmacher (1951). In lizards and snakes there is no epithelium in this string, which consists exclusively of portal vessels and connective tissue. It is then called *pars terminalis*, although Gisi(1907) used this term for the structure in *Sphenodon*, which contains epithelial tissue".

The *pars tuberalis* is formed by cellular cords and follicles containing colloid and traversed by connective tissue. The cells are usually chromophobic but there may be chromophil cells.

Cameron and Foster(1972) described two main types of cells in the rabbit by electron microscopy. The commonest type of cell contains 100nm cytoplasmic granules and plenty of polyribosomes. The second type consists of interstitial cells having elongated processes which encircle the first type of cells.

Gonadotrophic function was ascribed to this zone by Berblinger(1941) and TSH cells were noted by Kutas(1958). This zone may synthesize ACTH in dogs (Finerty and Keller, 1961). Fand and Thorell(1962) found TSH, FSH, LH and possibly ACTH cells in this zone by histochemical methods. In man some cells of the *pars tuberalis* have reaction with human chorionic gonadotrophin antibodies (Midgley,1963). It was concluded that some LH cells were present. Midgley(1966) and Dubois(1970) had evidences to suggest that LH cells are present in the *pars tuberalis* of human and bovine pituitary glands. Immunohistochemically LH gonadotrophs were found in groups in the *pars tuberalis* and they continued upto the superior surface of the anterior lobe. In the *pars distalis* these cells were single and not in groups and the extent of staining was also less compared to those in the *pars tuberalis*. Legait(1969) injected prepubertal rats (male and female) with homogenates of *pars tuberalis* and found a decrease in the volume of the *pars tuberalis*, stimulation of testicular interstitial tissue in male and of ovarian luteal tissue in females and concluded that *pars tuberalis* secretes LH. In the rat *pars tuberalis* consists of two parts (Legait and Contet, 1969). Gonadotrophs are in excess in one part, whereas in the other part the preponderant cell types are acidophils and chromophobes. After castration or



during gestation the gonadotrophs hypertrophied with the increase in the volume of the pars tuberalis. Ultrastructurally, the remnant of pars tuberalis after hypophysectomy in rats contained FSH and ACTH cells (Stutinsky *et al.*, 1964).

Costoff(1973) found STH, LTH, gonadotroph-like (LH) cells and few TSH cells and chromophobes in the pars tuberalis of the rat. He says, "It appears that the pars tuberalis might serve an important function in governing the gonads and even producing ACTH but these postulations remain to be established".

#### *Pars intermedia*

It is found in most mammals but not in all (fig. 6.7). It is present in amphibians and reptiles and absent in birds. The function of MSH is known in amphibians and fishes but its function in mammals is little known. Legait(1964) and Wingstrand(1966) reviewed the pars intermedia in greater details.

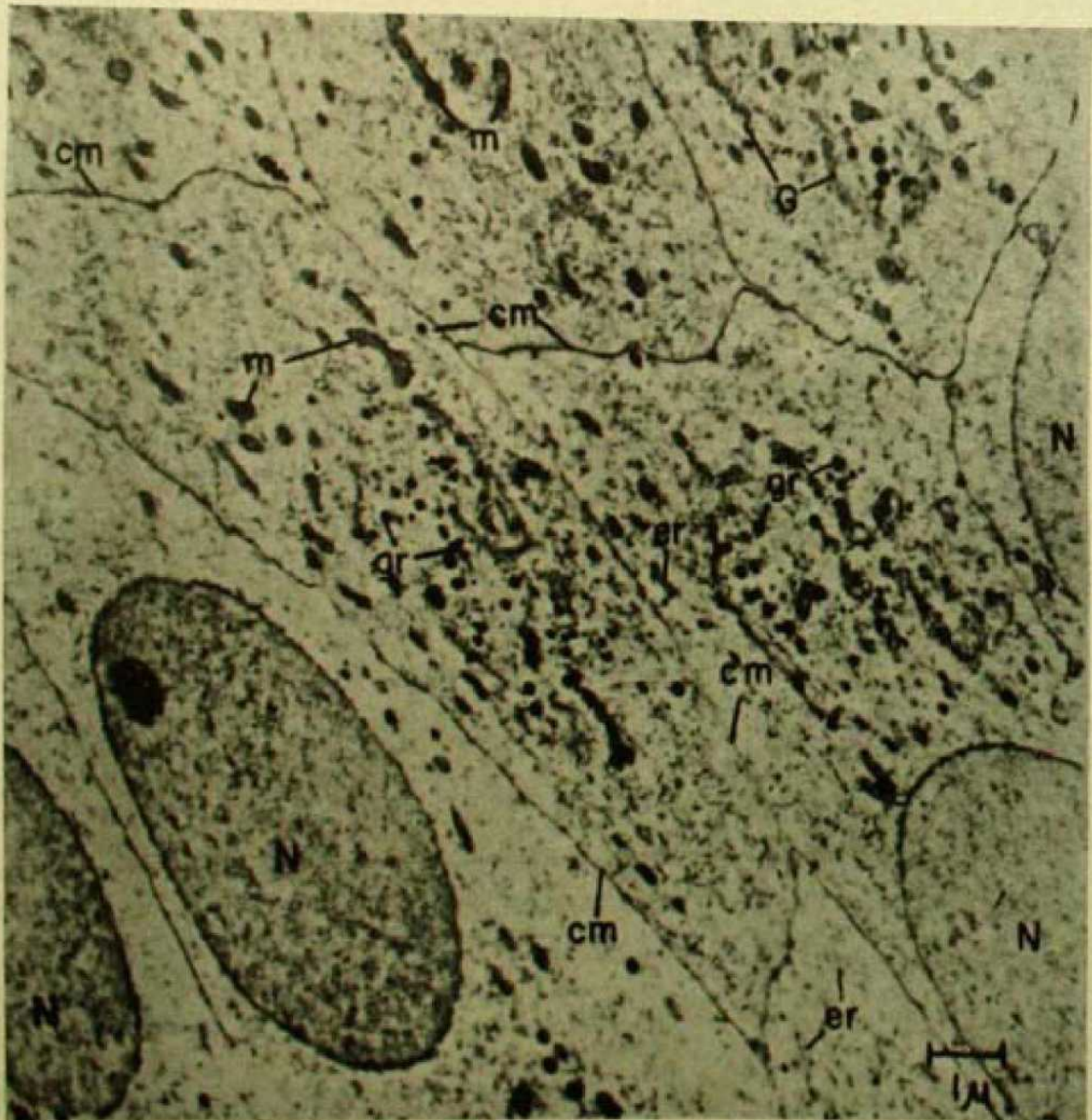


Fig. 6.7



Purves(1961) diagrammatically represented conventionalized mammalian hypophysis showing the divisions, components and parts. The pars tuberalis is formed by the adeno-eminence and adeno-stalk. The hypophysial cleft divides the adenolobe almost completely into an anterior lobe and an intermediate lobe (figs. 6.8, 6.9). In many mammals the anterior lobe is entirely composed of pars anterior tissue and the intermediate lobe is entirely composed of pars intermedia. In most mammals and in most other terrestrial vertebrates (except birds), Rathke's pouch adheres to the neural component during development. In the adult, Rathke's pouch persists as hypophysial cleft and so the adenolobe can easily be separated into two parts. The intermediate lobe is that portion of the adenolobe which is adherent to the neural lobe. The posterior lobe is formed by the adherent neural and intermediate lobes. The remaining portion of adenolobe is the anterior lobe. During development obliteration of the cavity of Rathke's pouch occurs in whales and porpoises and in the armadillo, manatee, elephant, pangolin, beaver, and the whole class of birds, and so there is no hypophysial cleft. Adenolobe is not adherent to the neural lobe in all these animals and these two structures can be easily separated from one another. "This is not a separation into anterior lobe and posterior lobe, which terms are not applicable to this type of hypophysis".

The pars intermedia (a part of the adenolobe adjacent to and adherent to the neural lobe) in some species is co-extensive with the intermediate lobe. In some (the sheep and the ox) it is less extensive than the intermediate lobe and the remainder of this lobe consists of pars anterior tissue. "Such a detached portion of the pars anterior is known as a *cone of Wulzen*". In the cat "the pars intermedia is more extensive than the intermediate lobe, and extends somewhat into the anterior lobe".

The *cone of Wulzen* is found in the pituitary of the ox and the sheep. Hanström (1966) said that this attribute is a remarkable prolongation of the pars intermedia into the hypophysial cleft. It possesses acidophils and basophils which are similar to the cellular population of the pars distalis. Hanstrom states, "On account of its position far from the embryonic neural lobe, the

Fig. 6.7 Electron micrograph showing a field of cells from the pars intermedia of the rat pituitary. The plane of section passes through the nuclei (N) of four of the cells, whereas only the cytoplasm of several other cells is transected. The cell membranes (cm) stand out prominently.

These cells are seen to fit together like a mosaic, and they all show a similar appearance. Their cytoplasm contains relatively few secretory granules (gr) and mitochondria (m). The endoplasmic reticulum (er) is present in the form of tiny vesicular profiles with occasional tubules. Elements of the Golgi complex (G) are visible in several areas. In these cells the dense, paired Golgi membranes and granules predominate over Golgi vacuoles, which are not seen at all in this field. X 9100. Courtesy of Professor M. G. Farquhar, Professor H. D. Purves, and the Williams & Wilkins Co. (1961).



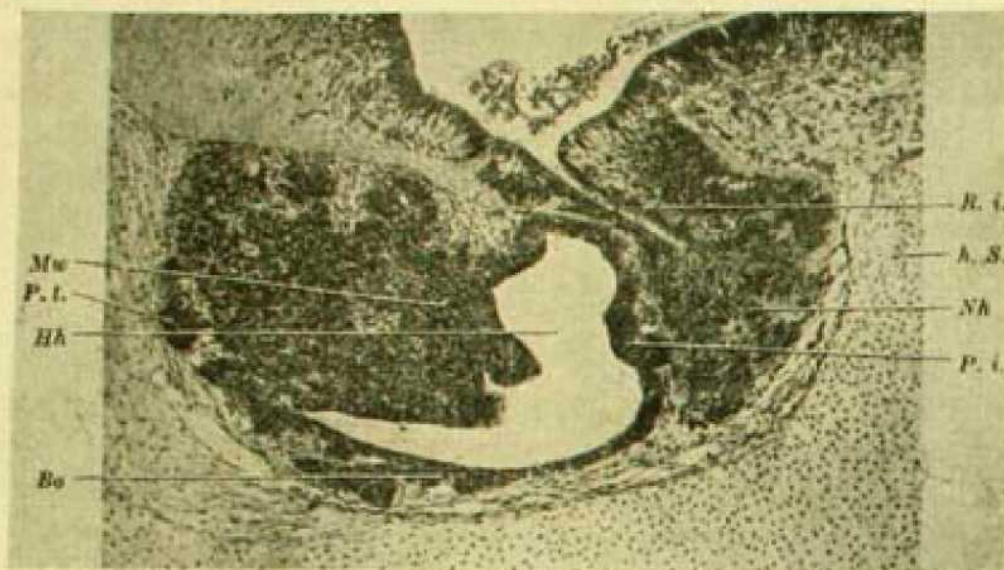


Fig. 6.8. Median sagittal section through the hypophysis of human embryo (44mm). The anlage of the pars intermedia has started to proliferate.

Bo = Floor of the hypophysial cavity ;

Hh = Hypophysial cavity (cleft) ;

Nh = Neurohypophysis ; P.i. = pars intermedia ;

P.t. = Anlage of pars tuberalis ; R.i. = Recessus

infundibularis. Bouin. Paraffin 10 $\mu$ .

Hemalum—eosin. Enlargement 1.75

From B. Romeis (1940). Courtesy of Springer-Verlag.

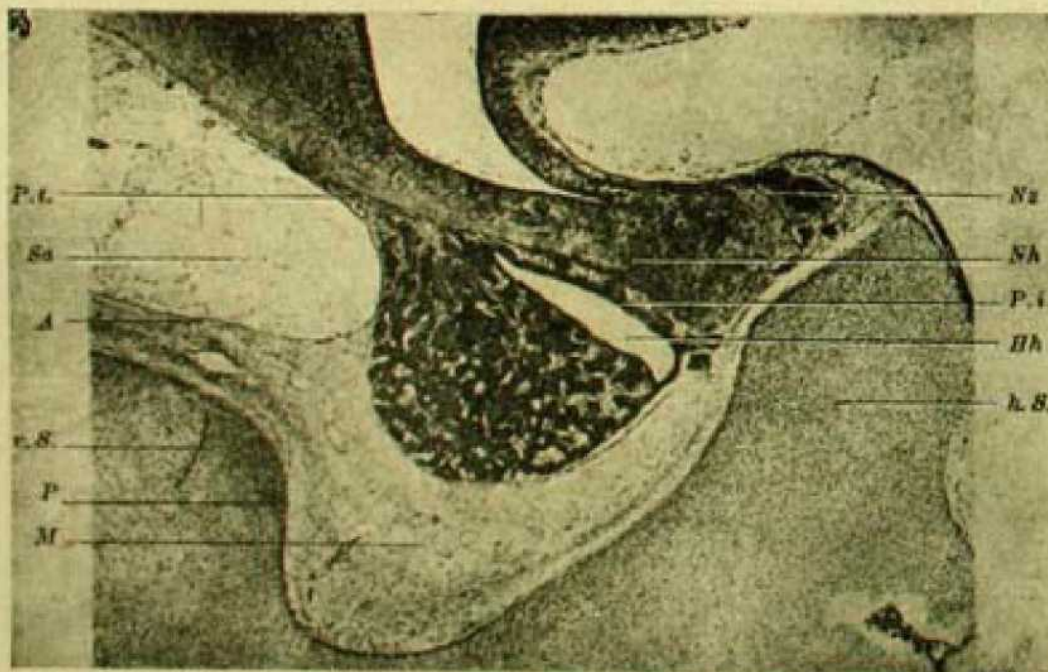


Fig. 6.9 Median sagittal section through the hypophysial region of human embryo (88mm).

A = Arachnoid ; Hh = Hypophysial cleft ;

Sa = Subarachnoideal space. Hemalum—eosin.

P = Perichondrium = Subsequent periosteum ;

P.i. = Pars intermedia ; P.t. = Pars tuberalis ;

Sa — Subarachnoideal space. Hemalum—eosin.

X 1:30. From B. Romeis (1940). Courtesy of Springer-Verlag.



peculiar histology of the Wulzen's cone may be explained by the fact that part of the connective tissue in the vicinity of the embryonic hypophysis invades the space between the neural and glandular lobes in the region of the cone and probably prevents the action of the formative influence of the saccus infundibuli on the region of the intermedia furthest from it. Consequently the cone does not assume the epithelial structure of the main part of the intermedia but develops histologically in the same direction as the pars distalis . . . . . The only instances of cone-like structures in other mammals are found in the pituitaries of the lesser panda (*Ailurus fulgens*) and the Florida manatee (*Trichechus manatus*) (Figs 6.10, 6.11, and 6.12).

There is no pars intermedia in man, whales, porpoises, armadillo, manatee, elephant, pangolin, beaver, and in the whole class of birds.

Purves(1961) studied the staining reactions of pars intermedia cells. The granulation in the cells is PAS + and so it appears to contain glycoprotein. It is also stainable by aldehyde-fuchsin without prior oxidation. The intermedia cell granulation is of glycoprotein character in the bat(Herlant, 1956) and in the frog (Ortman, 1954, 1956). In both the species the granules are aldehyde-fuchsin +. Similar staining reactions of the granules of pars intermedia cells have been observed by Purves(1961) in the cat, dog, sheep, deer, and rat. Intermedia cells are typical basophil cells having granules with a content of soluble glycoprotein. In the rat the granules are more aldehyde-fuchsin + than PAS +. With trichrome staining methods the granules are blue or purple (Romeis,1940).

In the human hypophysis intermedin was found in high concentration in the anterior lobe but the same was entirely absent from the neural lobe tissue. High concentration of intermedin was however, noted in areas of neural lobe having invasion of basophil cells (Morris *et al.*,1956). In human hypophysis intermedin is secreted by cells containing glycoprotein. These invading cells stain with aldehyde-fuchsin and take a red or purple shade from the trichrome counter stain. Purves(1961) stated, "In man this intermedin secretor is heavily granulated and stains strongly by PAS or by trichrome methods, as does the pars intermedia cell of the cat".

Wingstrand(1966) reviewed the microscopic anatomy, nerve supply and blood supply of the pars intermedia. Peremeschko(1867) described the pars intermedia as a white medullary zone(*Markschicht*) situated between anterior and posterior lobes of mammalian pituitaries. He used unstained thick sections. "In 1886 Lothringer characterized the mammalian intermedia as an *Epithelsaum*, indicating that it is a compact epithelial brim (or fringe) on the surface of the neural lobe"—Wingstrand(1966). The term pars intermedia was used by Herring(1908).

In many mammals and reptiles with a persistent hypophysial cleft the typical intermedia of the Lothringer type is a simple or stratified epithelium situated on the surface of the neural lobe and lobulation is found to be absent or very little.



COMPARATIVE ASPECTS OF THE PITUITARY GLAND

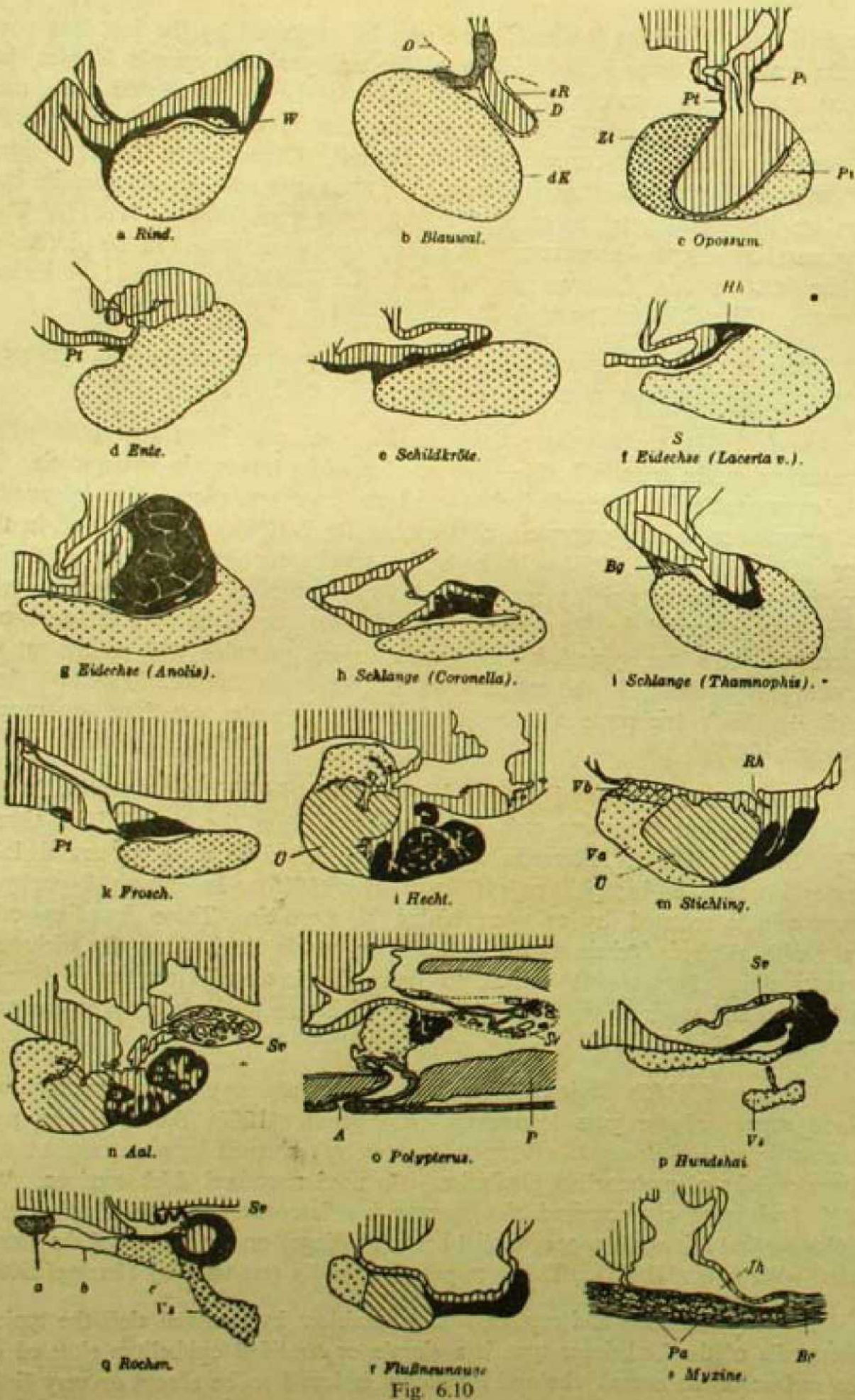


Fig. 6.10



Fig. 6.10 Schematic representation of median sagittal section through the hypophysis of different vertebrates. Coarse dot = Pars anterior; Fine dots = Pars tuberalis; Pars intermedia is black; The brain and its parts are vertically hatched and Übergangsteil is obliquely hatched. (a) *Ox*, after Atwel and Marinus (1918); W=Cone of Wulzen. (b) *Balaenoptera sibbaldii*, after Wislocki and Geiling (1936, Fig. 4e); D=Dura; dk=dural covering; sR=Subdural space. (c) *Opossum* after Dawson (1938, Fig. 3); Pi=Pars intermedia (thin epithelial lining); Pt=Pars tuberalis; Zt=Zona tuberalis. (d) *Duck* after de Beer (1926, Fig. 26).

(e) *Tortoise* after de Beer (1926, Fig. 39).

(f) *Lacerta viridis* (male). Hh=Rest of hypophysial cleft in the pars intermedia. S = Connective tissue filling up the space between anterior lobe on one side and derivatives of the brain and pars intermedia on the other. X 1:15.

(g) *Anolis carolinensis* after Poris and Charipper (1938, Fig. 1D)

(h) *Coronella austriaca* after Stendell (1914, Fig. 28).

(i) *Thamnophis radix* after Siler (1936, Fig. 1); Bg = Strings of connective tissue = Pars terminalis;

(k) *Rana esculenta* (female). Paramedian sagittal section through the pars tuberalis (Pt). X 1:15.

(l) *Esox lucius* after Sterzi (1904, Fig. 3);

Ü = Übergangsteil;

(m) *Gasterosteus aculeatus* after Bock (1928, Fig. 2);

Rh = Recessus hypophysius; Ü = Übergangsteil;

Va = Anterior lobe, chromophilic part; Vb = Anterior lobe, chromophobic part.

(n) *Anguilla vulgaris* after Stendell (1914, Fig. 41);

Sv = Saccus vasculosus.

(O) *Polypterus ornatipinnis* after Gérard and Cordier (1937, Fig. 2);

A = Opening of buccopharyngeal canal; P = Parasphenoid;

Sv = Saccus vasculosus.

(p) *Scyllium canicula* combined after Stendell (1914, Fig. 65) and de Beer (1926, Fig. 83); Sv = Saccus vasculosus; Vs = Ventralsac.

(q) *Raja* after Howes (1936, Fig. 3); a—c = Pars anterior. a = basophilic, b = chromophobic, c = oxyphilic zones; Sv = Saccus vasculosus; Vs = Ventral sac.

(r) *Petromyzon fluviatilis* after Stendell (1914, Fig. 18).

(s) *Myxine glutinosa* after Retzius (1895, Table VII, Fig. 1) and Stendell (1914, Fig. 40); Bg=connective tissue; Ih = Infundibular cavity; V1 = anterior lobe.

From B. Romeis (1940). Courtesy of Springer-Verlag.



# COMPARATIVE ASPECTS OF THE PITUITARY GLAND

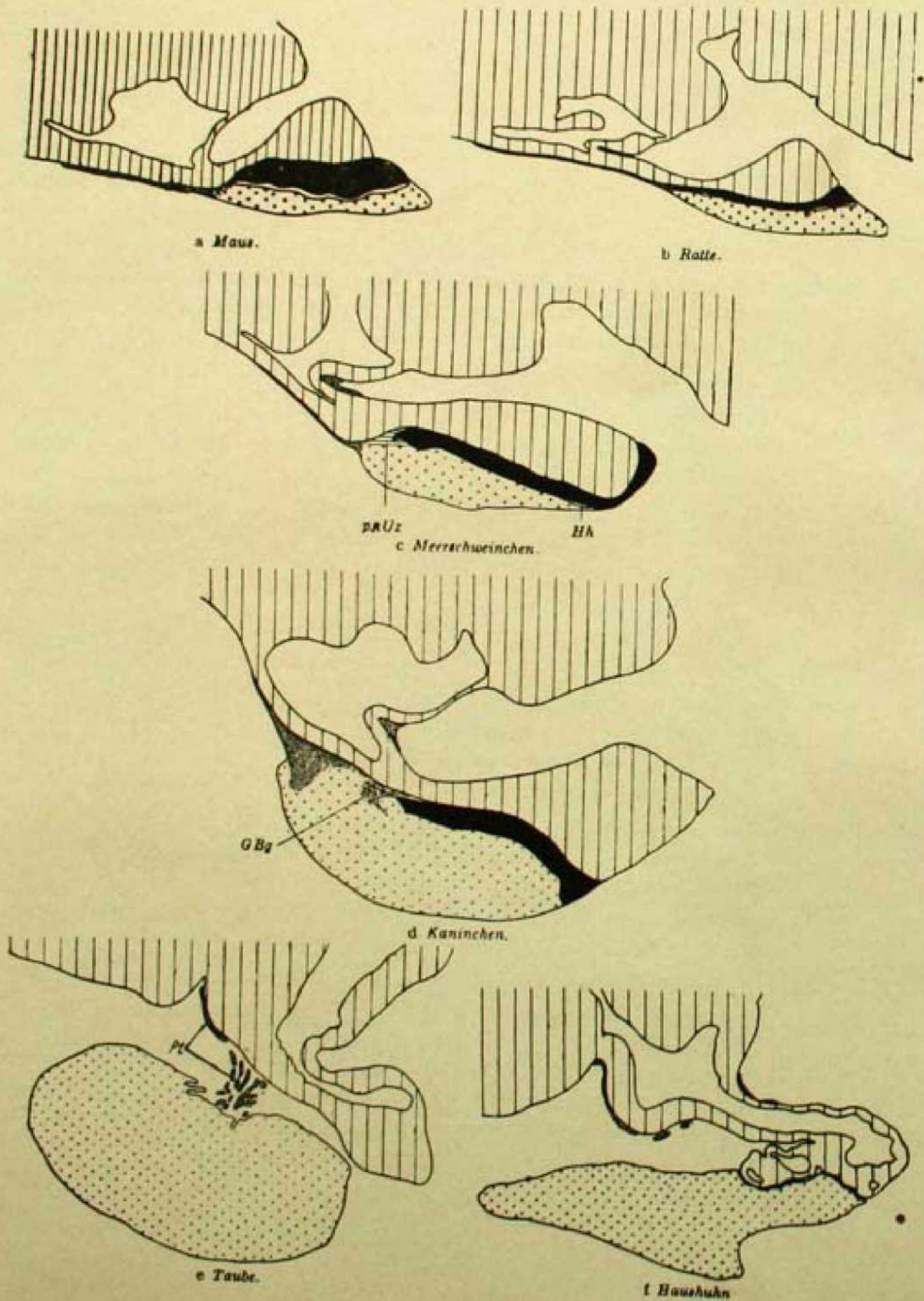


Fig 6.11



Fig. 6.11. a-f. Semischematic sagittal section through the hypophysis of different laboratory animals. Coarse dots = Pars anterior. Fine dots = Pars tuberalis. Black = Pars intermedia. Vertical hatchings = Brain and its derivatives.

(a) *Mouse*, aged 1 yr, male. X 1:35.

(b) *Rat*, 1 yr, male, X 1:13.

(c) *Guinea pig*, 1½ Yrs, male. Hh = Rest of hypophysial cleft; pnUz = paraneural Umschlagszone. X 1:13.

(d) *Rabbit*, 2 Yrs, male. GBg = Area containing vascular and connective tissue. X 1:13.

(e) *Pigeon*, 1 Yr, male. Pt = Pars tuberalis. X 1:21.

(f) *Cock*, 2 Yrs, male. X 1:13.

From B. Romeis (1940). Courtesy of Springer-Verlag.

Fig. 6.12. Schematic median sagittal section through the hypophysis of different groups. Pars intermedia is absent in 1-4.

(1) *Bird* hypophysis—example—*Garrulus glandarius*;

(2) *Tachyglossus setosus* (*Monotremata*, rudimentary pars intermedia. After Wingstrand and Hanström, 1951);

(3) *Whale* hypophysis (*Tursiops*; after Geiling, 1936);

(4) *Indian Elephant*; Znv — Zona neurovasculosa;

There are three different hypophysial types according to Smith-Agreda and Spatz. In the 1st and 2nd types the pars intermedia is seen on the ventral aspect of the posterior lobe. In the third type the pars intermedia is retracted from the surface of the posterior lobe so that its posterior pole is free. In the first type the pars tuberalis is asymmetrical. The infundibulum, infundibular stalk and posterior lobe is horizontally distributed. The posterior lobe is situated above the anterior lobe. In the third type the hypophysis is relatively small and the Pons is free. The anterior lobe in the second type lies ventro-orally whereas the posterior lobe lies dorso-caudally. In the third type the anterior lobe is situated anteriorly, the posterior lobe is posteriormost and the pars intermedia is in between.

(5) *Sorex*; (6) *Erinaceus europaeus*; (7) *Galemys pyrenaicus*;

(8) *Tupaia glis*; (9) *Nycticebus coucang* (*Prosimiae*);

(10) *Callithrix jacchus* (*Hapale*); (11) *Macaca mulatta*;

(12) and (13) = *Chimpanzee* and *Orang-utan* (Köhne, 1944 and Hanström, 1957); (14) *Homo sapiens*. To note the pars intermedia which is rich in cysts (Zona intermedia) (12 and 14). N = Nackenhypophyse (cytologically belongs to the anterior lobe). The hypophyses of 8 and 10 belong to the second type and those of 9 and 11 to the third type of Smith-Agreda.

From R. Diepen (1962). Courtesy of Professor R. Diepen and Springer-Verlag.

(Fig. 6.12 on reverse)



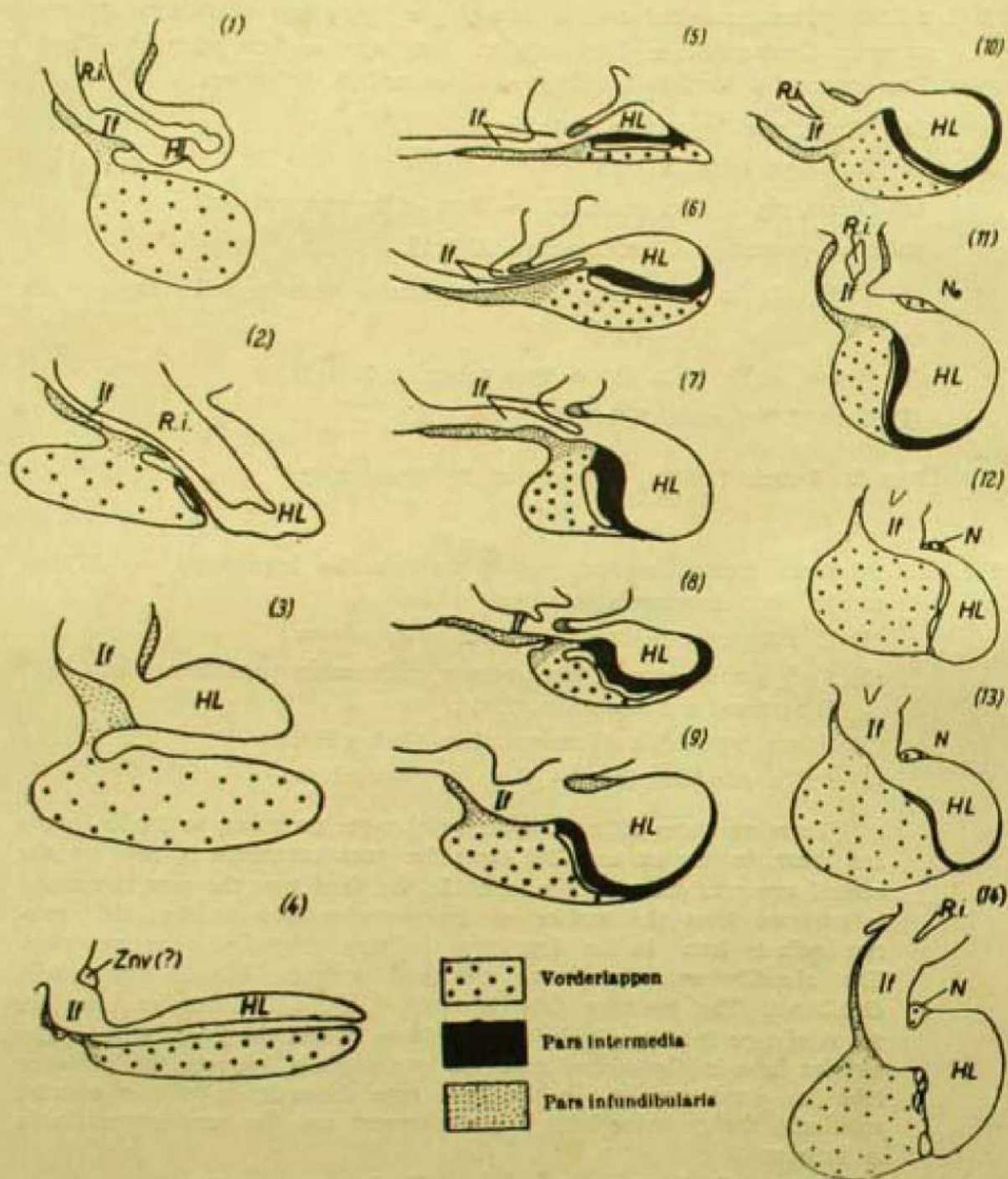


Fig. 6.12



The basement membrane, capillaries and connective tissue fibres are situated between the intermedia and the neurohypophysis. The *plexus intermedius* of Benda is attached to the basement membrane. In simple type (Lothringer type) capillary loops or arches do not usually penetrate into the intermedia from this basal plexus but they are more frequently noted in thick or lobulated intermedias. Single layered intermedia has been found in the gecko *Tarentula* by Wingstrand and also in some marsupial mammals. In mammals, lacertilians and in some chelonians the stratified intermedia consists of two or more cell layers. The *marginal zone* faces the cleft. In the rat the cells of this zone are non-granulated, flattened or cubical. Wingstrand (1966) found similar feature in the lizard *Anguis*. In carnivores (dog, cat and fox) the cleft-cells are ependymalike and are attached to the basement membrane (Romeis, 1940). The smooth juxta-

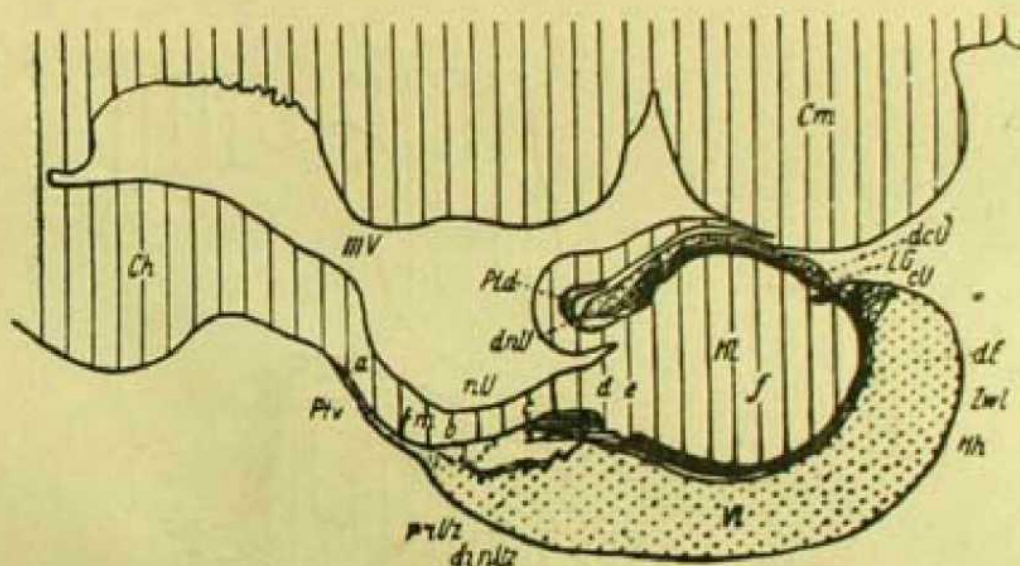


Fig 6.13. Median sagittal section through the hypothalamo-hypophysial region of a dog.

Ch = Chiasma; Cm = Region of mamillary body;  
cU = Ventro-caudal Umschlag; dcU = Dorso-caudal Umschlag;  
dnU = Dorsal nasal Umschlag;  
dinUz = Distal part of ventral nasal Umschlagszone;  
Em = Median eminence of the tuber cinereum; Hh = Hypophysial cleft; Lg = Gap for the entrance of the Inferior Hypophysial Artery; nU = Ventral nasal Umschlag;  
pnUz = Paraneural part of the ventral nasal Umschlagszone;  
Ptd = Dorsal part of pars tuberalis;  
Ptv = Ventral part of Pars tuberalis;  
VI = Anterior lobe; Zwl = Intermediate lobe; The anterior lobe is with coarse dots. The pars tuberalis is with fine dots. The pars intermedia is black. Dog (male) aged 1½ Yrs and 12 Kg. in weight. X 1:9.  
From B. Romeis (1940). Courtesy of Springer-Verlag.

neural side of the intermedia forms lobulations in many mammals specially in the rostral part of the intermedia (the *pars intermedia accessorius* of Hanstrom) having contact with the infundibular stem. This is a very constant feature in these mammals and the lobules are separated by low connective tissue septa, which cut into the epithelium from the basement membrane. The proliferated



zone is also known as *nasale Umschlagszone* or *zona rostralis* (figs 6.13, 6.14a, 6.14b). The pars rostralis of the rat is more vascularized and more lobulated than other parts of the intermedia and it rapidly responds to external stimuli.

Stratified intermedias with no or moderate lobulation have been noted in many *mammals* (*Ornithorhynchus*, insectivores, *Tupaia* and primitive primates, many rodents, some *Xenarthra*, and many carnivores) and many *reptiles* (*Sphenodon*, many lizards and some chelonians).

Nonlobulated intermedia is found in *Amphibians* and the cyclostome, *Petromyzon*. The intermedia is practically nonvascular. The compact intermedia is surrounded by basement membranes, covered by a plexus of capillaries or sinusoids.

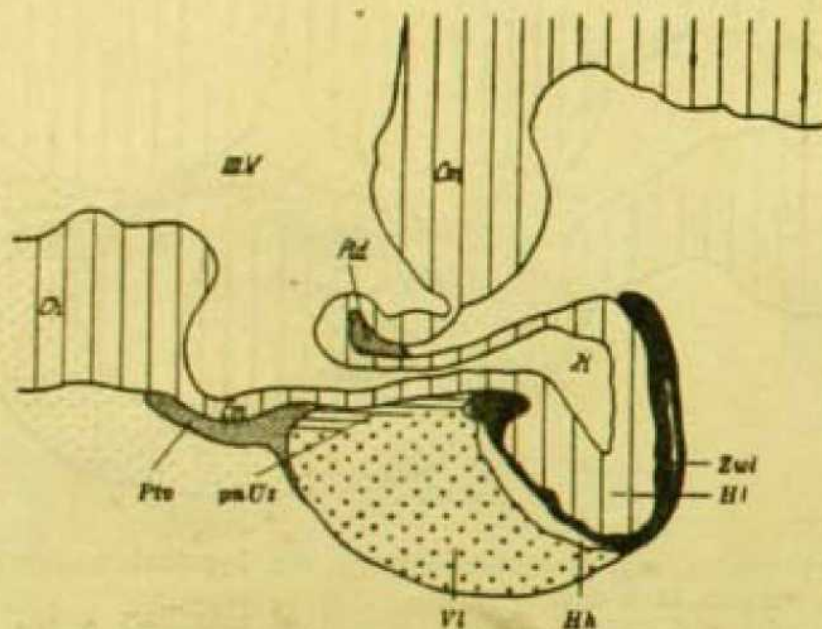


Fig. 6.14a. Median sagittal section through the hypophysis of an adult cat. Ch = Chiasma; Cm = Region of the mamillary body; Em = Median eminence of the tuber cinereum; Hh = Hypophysial cleft; Hl = Neural lobe; pnUz = Paraneural Umschlagszone; Ptd = Dorsal part of pars tuberalis; Ptv = Ventral part of tuberalis; VI = Anterior lobe; Zwl = Intermediate lobe; The figure is from a median sagittal section through the hypophysis of a noncastrated cat (3 yrs). X 1:9. From B. Romeis (1940) Courtesy of Springer-Verlag.

*Extensive lobulation of the intermedia* has been noted in some mammals, reptiles and fishes. The lobules are separated by septa formed by basement membranes, connective tissue and capillaries. Wingstrand noted proliferation from the neural side of the intermedia in some lizards with a persistent cleft e.g. *Varanus*. Thick lobulated intermedias have been found in Lungfishes (Wingstrand). The lobules have lumina communicating with the persisting hypophysial cleft. The neurointermediate lobe (metaadenohypophysis) of teleosts



and chondrichthyes (sharks and rays) has compact lobules, intermingling with the processes of the neurohypophysis. Intermingling between the intermedia and the pars distalis is frequently seen in mammals with an obliterated hypophysial cleft.

The membrane between the intermedia and the neural lobe shows some defects in many vertebrates and the intermedia cells spread into the neural lobe through these openings. This is a normal feature of the human pituitary. Such invasion has also been found in most orders of mammals. Legait and Legait (1962) thought that intermedia cells actively invade the neural lobe in some rodents in response to osmotic stress.

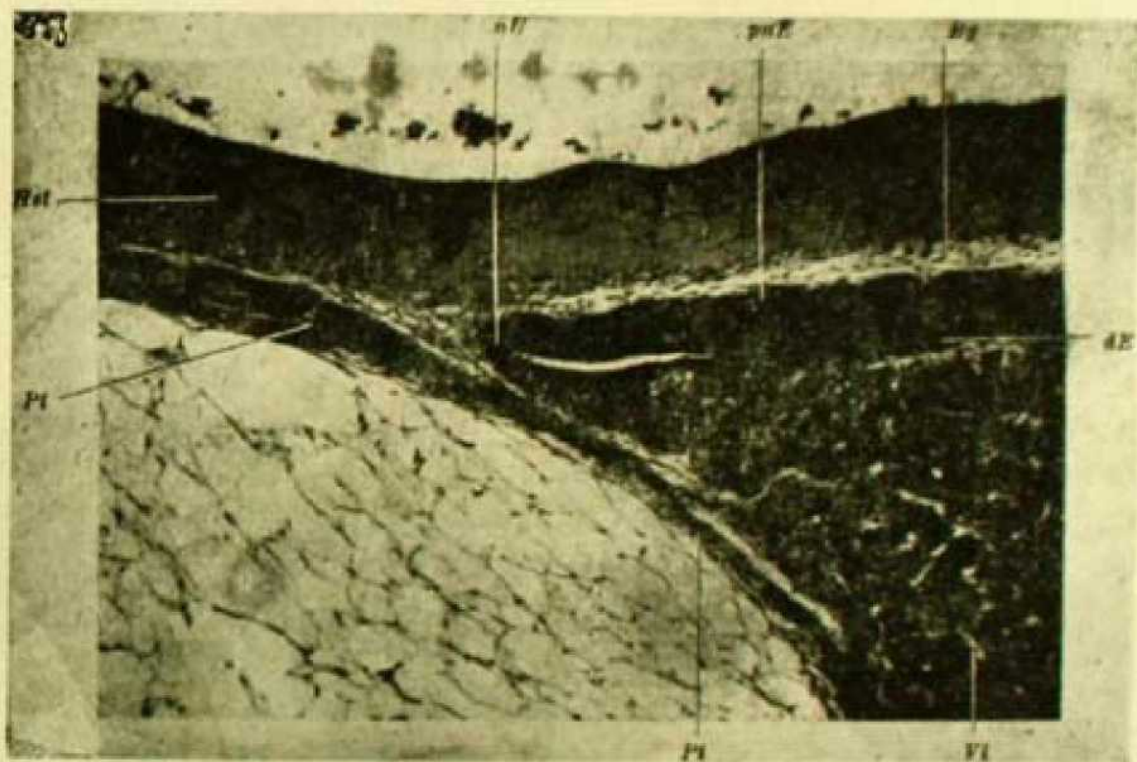


Fig. 6.14b. Median sagittal section through the nasal part (nasalen Teil) of the hypophysis of a kitten. Bg = Connective tissue strip; dE = Distal epithelial lining of the hypophysial cleft; Hst = Hypophysial stalk. nU = Nasal Umschlag of the hypophysial cleft; pnE = paraneural epithelium of the hypophysial cleft; Pt = Pars tuberalis; VI = Anterior lobe. Male kitten, aged 8 days. Paraffin 15 $\mu$ . Hemalum-Eosin. X 1:102.

From B. Romeis (1940). Courtesy of Springer-Verlag.

Wingstrand(1966) stated, "The intermedia of the *embryonic human pituitary* is a typical, multilayered epithelium on the neural lobe, separated from the pars distalis by a cleft. After birth, the cleft is usually reduced to a zone of discontinuous lumina, the Rathke's cysts. The epithelial cover on the neural lobe undergoes regressive development, so only a thin, discontinuous layer, one or a few cells thick, is present in the *adult*. The zona rostralis is evaginated into several hollow lobules in the embryo but is reduced in the adult to a group of small cysts in the angle between the neural lobe and the infundibular stem.



The limits of this vestigial human intermedia fade out by extensive intermingling with the pars distalis and by invasion into the neural lobe. Therefore, a distinctly delimited pars intermedia can hardly be recognized in the adult human pituitary, and the majority of recent authors follow Romeis'(1940) suggestion and call the modified zone along the neural lobe *Zona intermedia*."

The human intermedia shows the following features. These are indicated in the higher primates but rare in the majority of mammals (Hanstrom, 1957): (1) extensive invasion of basophilic cells into the neural lobe; (2) frequent occurrence of different kinds of cysts; (3) development of tubular glands; and (4) high incidence of lymphoid infiltration. The cysts were classified by Romeis into four different types: (1) Rathke's cysts; (2) cysts formed by evaginations of the zona rostralis; (3) glandular cysts; and (4) pseudofollicular cysts.

Two types of cells were identified by Lathringer (1886) in the intermedia of the dog: (1) rounded glandular cells; and (2) slender, ependyma-like cells. Romeis(1940) called the slender cells *undifferentiated intermedia cells*. The *marginal cleft cells* in rodents are also undifferentiated cells according to Romeis. He distinguished the hyperchromatic dark staining cells from the common intermedia cells.

#### *Innervation*

In the mouse nerve fibres entered the intermedia from the lobus nervosus and ramified among the glandular cells (Ramon y Cajal, 1894). Similar feature was subsequently found in different mammals and man. Different types of intra and extracellular nerve endings in the form of knobs, bulbs and pericellular nets were observed with silver techniques. Neurosecretory fibres and neurosecretory material(nsm) are found between the cells of the mammalian intermedia. Electron microscopic studies have subsequently proved the presence of fibres with typical neurosecretory granules and bulbs(as seen in silver preparations) (Kurosumi *et al.*, 1961.)

Intermedia was found to be innervated in non-mammalian vertebrates in silver preparations and after using stains for neurosecretory material. The nerves are said to be inhibitory in action. Increased MSH secretion and blackening of the animal associated with increased growth has been found after lesions or transplantations leading to interruption of the hypothalamic innervation of the amphibian intermedia. Recent experiments prove the presence of secretory nerves which increase MSH secretion (Jorgensen and Larsen, 1960).

#### *Vascular supply*

The plexus intermedius of Benda is situated in the membranes between the neural and intermediate lobes of mammals and reptiles and mainly or exclusively supplies the compact intermedia. The internal capillaries of the intermedia are few in number and are situated in the interlobular space. The zona rostralis of the mammalian intermedia has a comparatively rich blood supply in the rat, the dog and the cat.



Because of the poor vascularization hormones secreted by the deeply situated cells, pass out through other cells or through the intercellular spaces. The arterial supply of the common posterior lobe plexus is by branches of superior hypophysial arteries coursing down the infundibular stem or inferior hypophysial arteries entering from behind. In sharks additional supply to the intermedia is through portal vessels coming from the median eminence (Meurling, 1960).

Wingstrand(1966) said, "The venous drainage is also, in general, identical with the venous drainage of the neural lobe. Overflows from the neuro-intermediate plexus to the pars distalis are common along the periphery of the intermedia, where it is connected with the pars distalis. In mammals they are of three different kinds: (1) vessels running from the posterior lobe plexus to the pars distalis in front of the anterior margin of the intermedia, parallel with the portal vessels; (2) vessels which pass around the anterior and lateral margins of the intermedia, following the surface of the adenohypophysis; and (3) vessels which run through the hypophysial cleft at such places where its two walls have fused to form a bridge". These overflows can save the adjoining parts of the pars distalis from necrotic disintegration in case the normal blood supply to the distalis is jeopardised experimentally or by pathological processes (Daniel and Prichard, 1956, 1957, 1958; Holmes and Zuckerman, 1959; Holmes, 1962; Smith-Agreda, 1962).

#### *Costoff's observations:*

Regarding the composition of pars intermedia in the rat, Costoff identified three zones: (1) Chromophobic cells (1-3 layers) line the residual hypophysial cleft (*cleft cells*), (2) about twelve layers of cells comprising light and dark chromophil cells of the central part of the pars intermedia and (3) dark cells face the pars nervosa and axonic end bulbs contain neurosecretory granules. These granules most likely originate from pars nervosa.

Cleft cells are ciliated chromophobe-like. Ferrer(1956) found an increase in the size of the cleft and amount of the colloid after adrenalectomy and castration. After estrogen, testosterone, or cortisone treatment the cleft was reduced in the rat and there was no colloid (Vanha-Perttula,1966). Costoff found the agranular cleft cells to have vesicular endoplasmic reticulum, a Golgi complex, round or rod shaped mitochondria with broken cristae, free ribosomes and lysosomes. Cleft cells are attached to each other by desmosomes. Oftenly the basement membrane is absent. Basophilic or acidophilic cells may sometimes be found between the cleft cells on the side of the pars distalis. The apical surface of the cells contains microvilli and cilia with 9+2 fibre pattern.

Ciliated acidophils and gonadotrophs have also been found in the anterior pituitary. The pattern of the cilia is 9+0.

Iturriza and Koch(1964) thought that the colloid in the cells is a storage form of MSH and bound to glycoprotein. Vanha-Perttula(1966) noted broken



cells from pars distalis in the colloid of the cleft. It was glycoprotein in nature. The colloid is the neurosecretory material according to Kurosumi *et al.* (1961, 1962). The cleft also contains secretions from pars intermedia dark cells. The colloid contains ACTH from the pars distalis or pars intermedia, CRF or other nsm

*Cells at the central part of the pars intermedia :*

Light and dark cells in the pars intermedia were described by Trautman (1911). Romeis (1940) and Kurosumi *et al.* (1962) observed the same in man and rat respectively.

The predominating cell type is light Type 1 cell. It is weakly PAS+ compared to strong PAS+ Type 2 cell (Purves, 1961). The carrier for MSH gives rise to PAS+. *Type 1 cells* (Costoff) are plenty in number (70%). They are cuboidal with large round nucleus. Mitochondria are round or short rods with incomplete cristae. The Golgi complex is inconspicuous. RER is vacuolar and there are free ribosomes. Lysosomes are present. Granules (300-350 m $\mu$ ) are situated at one pole and near the cell membrane.

*Type II dark cells :* They are few in number and the shape is angular or stellate shaped. The granules are 200 m $\mu$  in diameter and are located throughout the cell. Some cells have large dilated areas of endoplasmic reticulum studded with ribosomes. Rodlike mitochondria have complete cristae. Golgi complexes are very few.

Small chromophobe-like cell is sometimes found. The dense cytoplasm is scanty and the nucleus is large. The granules or dense bodies are 250 to 300 m $\mu$  in diameter and there are free ribosomes. Costoff said that this cell type corresponded to the rhomboid cell of Kobayashi (1965).

*Pars intermedia towards the pars nervosa :* Ramon y Cajal (1894) observed nerve fibres extending into the pars intermedia from the pars nervosa. Near the neural lobe, pars intermedia had axons and type 1 cells. There are two types of nerve fibres: one can be demonstrated by metallic impregnation techniques and the other one is neurosecretory. Neurosecretory and aminergic nerve fibres (fluorescent technique) have also been detected. Ultrastructurally neurosecretory nerve fibres enter from the neurohypophysis into the mammalian pars intermedia, coming in close contact with the cells (Kurosumi, Matsuzawa and Shibasaki, 1961; Ziegler, 1963). Fibres containing dense cored vesicles (100nm) enter this part of the gland and they are associated with catecholamines.

After stress (formalin injection) and adrenalectomy there were ultrastructural changes in the cells of the pars intermedia (Kobayashi, 1965). Granules increased in number in the light Type 1 cells. The granules had similarity with the neurosecretory vesicles of axons and the suggestion was that there is a transition from 350 m $\mu$  granules to 200 m $\mu$  vesicles in Type 1 cells.



By autoradiography Gosbee *et al.* (1970) found relationship between ACTH increase in granulation, activity After adrenalectomy stellate Type II cell had increase in granulation, activity and colloid. No increase in MSH concentration in the pituitary and plasma could be found but there was an increase in ACTH. After adrenalectomy Type II cells synthesized ACTH. Phifer and Spicer (1970) found ACTH in the pars intermedia of different species of animals including the rat by using immunoglobulin-peroxidase methods. Corticotrophs were found in the pars intermedia of the rat (Porte *et al.*, 1971). More ACTH was found in the rat pars intermedia than in the pars distalis (Kraicer *et al.*, 1971).

In the pituitary autografts to the kidney capsule there was synthesis and secretion of MSH in Weasels (Rust and Meyer, 1968).

Costoff (1973) thought that a pro-ACTH or ACTH might also be synthesized in the cells of the pars intermedia after stress and "perhaps the type II cells synthesize ACTH under these conditions".

### Lysosomes

Kurosumi (1974) while discussing about lysosomes in somatotrophs said that these bodies are located in the cytoplasm and have acid hydrolases. Lysosomes may be primary or secondary. The primary lysosome (pure lysosome) contains enzymes and also their substrates. The Golgi apparatus or its associated tubular system gives rise to the primary lysosomes and these are called coated vesicles because the vesicles are often covered with bristle-like coating. Intracellular digestion with the help of enzymes derived from the primary lysosomes takes place in secondary lysosomes. The substrate may come either from the interior of the cell or from outside the cell. On the one hand they are carried into phagocytic vacuoles by phagocytosis or pinocytosis (together called as *endocytosis*) from outside the cell, while on the other hand autophagic vacuoles are produced by sequestration of some of the cell organelles or certain products in the cell. Multivesicular bodies are frequently considered as secondary lysosomes. These bodies are vacuoles of different sizes containing small vesicles and appear in close approximation to the Golgi apparatus and it is possible that a transition exists between the multivesicular bodies and lysosomes. They contain secretory granules of the somatotroph. Secretory granules in these bodies are digested by hydrolytic enzymes. This is Farquhar's *crinophagy* noted in prolactin cells where overproduction of prolactin granules is disposed after sudden weaning of the mother (rat).

Apart from the vesicles, the secondary lysosomes may have myelin-like lamellar structures, droplets, particles or crystalloids. Secondary lysosomes are also called dense bodies because of their very high electron density. After adrenalectomy somatotrophs possess many secondary lysosomes.

Near the well developed Golgi apparatus of thyroidectomy cells there is formation of a complicated glomerular network of branching and anastomosing



tubules. Their origin either from the lamellated structures of the Golgi apparatus or from its associated tubular structure has not yet been established. There is accumulation of a dark substance in these tubules of different shapes. These bodies resemble the lysosomes. They contain acid phosphatase and Kurosumi (1974) thought that they may contain other hydrolytic enzymes too which occur in lysosomes. The formation of primary lysosomes may induce the formation of secondary lysosomes some time after.

Another characteristic change occurs in the thyroidectomy cell. Dilatation of rough-surfaced endoplasmic reticulum is found with accumulation of colloid-like substance. In these dilated spaces, round and dense granules have been noted by Kurosumi and Oota (1966). The size of these intracisternal granules varies from 100 to 500 nm in diameter. These are due to stimulation of protein synthesis in the thyroidectomy cells by TRH of the hypothalamus. Lysosomal substances are stacked in the Golgi apparatus and so transfer of the granules from endoplasmic reticulum to Golgi apparatus cannot take place and thus the intracisternal granules increase in number. At the same time many secondary lysosomes occur in the cytoplasm.

#### *Discharge of granules from adenohypophyseal cells:*

##### *Corticotroph:*

Kurosumi (1974) stressed that fixation of the specimen with plain osmium tetroxide solution is a more useful method for study of the morphology of corticotrophs than a fixation with mixed or serial fixation with glutaraldehyde and osmium. With osmium fixation most of the secretory granules are found as vesicles with a dense core; some granules take the appearance of clear vesicles and some are medium dense granules. A few granules only are of high electron density. Other cell types in the anterior pituitary do not contain such cored vesicles. After fixation with glutaraldehyde all secretory granules of corticotroph look black.

After adrenalectomy some cored vesicles are noted after osmium fixation. Kurosumi (1974) speculates that such a picture may be due to a process of granule discharge. The cortical part of the granule is dissolved and thus the granule core slips out to the extracellular space. Though Kurosumi's type IV, eruptocrine mechanism or exocytosis is found in the corticotroph, diacrine or type V secretion can also occur. There is probability of leaking out of some substance from the secretory granule across the limiting membrane and also the plasma membrane.

##### *Thyrotroph:*

There is fusion of the limiting membrane of the secretory granule and the surface plasma membrane of the cell and a small pore is formed at the point of fusion. The interior of the granule slips out through this pore. This is erup-



ocrine secretion, exocytosis, reverse pinocytosis or Kurosumi's type IV mechanism.

### *Somatotroph :*

The discharge of the secretory granules is by the method described above (exocytosis).

### *Mammotroph (estrogen-induced pituitary tumor) :*

The mechanism of the granule discharge is by exocytosis (reverse pinocytosis) or eruptocrine secretion. Kurosumi(1974) said "in a few cases a small invagination of membrane adorned with the bristle-like coating is observed at the surface of a secretory granule just opened to the extracellular space. Such an invagination of the granule membrane recalls the so-called pinocytosis seen in other kinds of cells. Such a phenomenon of micropinocytosis occurring on the releasing secretory granule membrane may contribute to the keeping the total surface area of the cell from its enormous increase by successive addition of secretory granule membranes to the surface plasma membrane".

### *Gonadotroph type 1 (FSH-cell) :*

Kurosumi(1974) found two types of secretory granules. One was large and less dense and the other small and dark in appearance. Small granules are plenty in number. The small, dark granules are 200-250nm in diameter. There is a limiting membrane. The large granules are of varied electron density and are of 500nm or more in diameter. Majority of these granules are less dense. A thin membrane surrounds the large secretory granule. After fixation with osmium tetroxide alone the limiting membranes of these large granules are ruptured and the granule is dissolved with formation of clear vacuoles or empty spaces without any limiting membrane. Such an artefact is not found in the small granules. After double fixation with glutaraldehyde and osmium the density of the large granule is increased and the density is same in both large and small granules. Both the types of secretory granules are finely granulated. This characteristic is found only in this cell type. The two types of secretory granules may be independent and contain different substances or they may be interrelated.

Presence of both the types of secretory granules is more marked in male rat pituitary gland than in the pituitary gland of a female rat. A few large granules only have been noted in female gonadotroph type 1 cell. Castration in male leads to a marked change in type 1 gonadotroph with disappearance of large granules. Type 1 gonadotroph secretes both FSH and LH but it is not known whether type 2 gonadotroph secretes also two different gonadotrophins.

### *Gonadotroph type 2 (LH-cell) :*

Kurosumi(1974) said that the secretory granules and other structural components of this cell type resemble those of growth hormone-secreting cells



(STH-cells). The size of the granules of type 2 gonadotroph is always smaller than that of the STH-cells. Thyroidectomy leads to marked degranulation of STH-cells but with this procedure the type 2 gonadotroph is indifferent. After castration intense change is noted in type 1 gonadotroph but the change is not so intense in type 2 gonadotroph. However, Kurosumi (1974) further stated that type 2 gonadotrophs are hypertrophic in every respect after castration with enlargement of the cell body, irregular shape and ER is well developed. Marked dilatation of the cisternae is not noted. The discharge of granules is by eruptocrine secretion (reverse pinocytosis).

#### *Release mechanisms of adenohypophysial hormones*

Kraicer(1975) presented a simple model of the physicochemical events that are associated with the release of adenohypophysial hormones. Roy(1976) reviewed such mechanism. The release process as presented by Kraicer(1975) is as follows: The process starts by an interaction between the hypothalamic releasing hormone and a specific receptor site on the plasma membrane of the cell. This results in an increase of prostaglandin synthetase activity with a subsequent increase in prostaglandin synthesis. Rise in prostaglandin activates membranebound adenyl cyclase which then increases cyclic AMP level within the cell. A change in the intracellular distribution of  $Ca^{2+}$  is elicited by cyclic AMP and it activates a cyclic AMP—dependent protein kinase. Alternatively, the activated protein kinase changes the intracellular distribution of  $Ca^{2+}$ . The protein kinase with  $Ca^{2+}$ , then specifically activates, by phosphorylation, a protein moiety involved in the release process. "The activated protein moiety may be one element in the contractile cytoskeleton-vesicle complex, which when activated, leads to contraction with subsequent extrusion of hormone-containing granules".

#### *Synthesis of anterior pituitary hormones*

Information proceeds from DNA of chromatin and arrives at ribosomes via messenger RNA(m-RNA) produced at the nucleolus. The releasing factor increases the amount of r-RNA directly or indirectly. Synthesis of proteinaceous secretory substance may be at ribosomes and this was studied by electron microscopy of exocrine glands (pancreas and salivary glands). Ribosomes are small granules which contain ribonucleic acid and protein measuring about 150Å in diameter. Two subunits of this granule have been recently reported. The ribosomes are usually found to be attached to the external surface of the endoplasmic reticulum(ER). Kurosumi (1974) described the sequence of events pertaining to somatotroph.

The secretory substance in the initial stage is accumulated within the rough-surfaced (ribosome-attached) ER. Subsequently the secretory substance may be transferred from the rough ER to the Golgi apparatus. The transport mechanism of the secretory substance from ER to Golgi apparatus may be by :—





- (1) direct continuity between the two (only noted in the neurosecretory cell of the hypothalamus but not found in the anterior pituitary)

and (2) a process called *budding or blebbing* which is frequently noted in the anterior pituitary gland. From the smooth surface of the rough ER which faces the Golgi apparatus there are formations of small nipple-like projections. The projections are pinched off and thus there are formations of many vesicles. The vesicles contain the secretory substance in the process of transport from the RER to Golgi apparatus. The secretory material (mainly protein) is condensed in the Golgi sacs with addition of a little sugar (polysaccharide) and lipid. These newly formed secretory granules are small in size and ill defined and float in the sacs of the Golgi cisternae. The secretory material increases in size and gradually the space between the granular material and the limiting membrane diminishes and ultimately vanishes. So, in the mature secretory granule of the somatotroph it is very difficult to separate the limiting membrane from the dark granule. The secretion mechanism in thyrotroph is same as noted in somatotroph.

#### *Immunoelectron microscopy*

Nakane (1975) discussed several problems related to the immunocytochemical localization of hormones in the anterior pituitary gland at the ultrastructural level.

- (1) The protein or glycoprotein hormones are soluble in usual physiological solutions and fixatives should make them insoluble.
- (2) Fixation of the hormones should be very rapid otherwise they will diffuse from their original place.
- (3) If diffusion occurs before fixation, the hormones will be fixed at secondary sites and thus a confusion will arise.
- (4) As the fixatives used in electron microscopy are directed towards proteins, it is expected that there is loss of antigenicity of hormone during fixation. Unsuccessful localization of a given antigen may be due to a) nonfixation of the antigen and it has been washed away during fixation or b) the antigen is there but it has been denatured during fixation. The effect of the fixative may vary according to the sites of the antigen. An antigen in the Golgi complexes is more easily affected than that in the endoplasmic reticulum.





*Identification of cell types in the anterior pituitary gland of adult albino male rats by immunoelectron microscopy (Nakane, 1975)*

	Growth hormone cells	ACTH cells
Shape	Oval to pyramidal	Stellate in shape
Size	Approximate diameter of 10-13 $\mu$ m	
Situation	Not found in areas adjacent to the intermediate lobe and anterior ventral portion of the gland but seen in other areas	Same as growth hormone cells. ACTH cells are in juxtaposition to growth hormone cells. The body of the cells is at the centre of a cord.
Relationship to sinusoids	Situated along sinusoids	The cytoplasm of the cell is extended to neighbouring sinusoids
The nucleus	Usually situated in the centre of the cell.	
Secretion granules	Diameter of about 300-350 m $\mu$ & situated throughout the cytoplasm.	Diameter of about 200 m $\mu$ & situated near the periphery of the cytoplasm. Some granules are solid and some have central cores.
Rough endoplasmic reticulum	Laminated. Found frequently near the nucleus.	Clustered near the nucleus
Golgi bodies		Clustered near the nucleus
Mitochondria	Found in clusters near the nucleus and sometimes among the secretion granules.	Clustered near the nucleus.





*Identification of cell types in the anterior pituitary gland of adult albino male rats by immunoelectron microscopy (Nakane, 1975)—(Contd.)*

	Prolactin cells	TSH cells
Shape	Frequently cup-shaped surrounding gonadotrophic cells.	Polygonal to stellate in shape.
Size		Larger than ACTH cells.
Situation	Sparsely distributed in the anterior ventral portion of the gland. Also seen in areas near the intermediate lobe.	Found in clusters in the centre of the pituitary gland. Cells situated in the centre of the cord like ACTH cells. Cell clusters did not contain ACTH cells and vice versa.
Relationship to sinusoids		The voluminous cytoplasm directly faced the wall of the sinusoids.
The nucleus	Situated centrally at the base of the cup.	
Secretion granules	Cytoplasm surrounding gonadotrophic cells also contained the hormone. The hormone was also found near the nucleus. Diameter of granules exceeded 800 m $\mu$ and varied from spherical to polymorphic in shape.	Granules containing TSH were situated at the periphery of the cell near the plasma membrane. Granules were 150—200 m $\mu$ in diameter. Some granules looked solid and some had central cores.
Rough endoplasmic reticulum	Well developed.	
Golgi bodies		Golgi complexes were situated near the nucleus.
Mitochondria		Round or oval mitochondria were found among large vacuoles. These vacuoles contained little or no hormone and were noted throughout the cytoplasm.



*Identification of cell types in the anterior pituitary gland of adult albino male rats by immunoelectron microscopy (Nakane, 1975)—(Contd.)*

	FSH Cells.		LH cells
	Tissue sections reacted with anti—FSH		
	Type A cells	Type B cells	
Shape	Oval	Angulated cells	Cellular morphology same as type A FSH cells
Situation	Distributed throughout the anterior pituitary gland and the area adjacent to the intermediate lobe. Concentrated in the sex zone.		Same as type A FSH cells
Secretion granules	Granules contained hormone. Situated among large vacuoles	Secretion granules situated near the plasma membrane.	Localization of LH was in several types of secretion granules in the cell. LH was found in small dense granules (75 to 250 m $\mu$ ) and in large dilated vacuoles (600 m $\mu$ in diameter)
	Secretion granules were 200—250 m $\mu$ in diameter.		
Rough endoplasmic reticulum	Dilated rough endoplasmic reticulum was situated throughout the cytoplasm.		
Mitochondria	Dispersed mitochondria were among the endoplasmic reticulum saccules.		



### *Autoradiographic studies*

Autoradiographic studies were conducted by Stumpf, Sar and Keefer (1975) for localization of hormones in the pituitary of the rat. There are receptor sites for hormones from hypophysial target glands and the brain. In the rat during pregnancy and lactation, topographically distinct cell population concentrates more radioactivity when compared to other labeled cells in the pars distalis. Gonadotrophs with heavy nuclear concentration of radioactivity after the injection of  $^3\text{H}$ -estradiol are noted in the sex zone and scattered heavily labeled cells are also found in other parts of the anterior lobe. Many anterior pituitary cells show less intense moderate nuclear labeling. There was a moderate uptake of radioactively labeled estrogen in the lining cells between anterior and intermediate lobes and in invaginated cells found in the intermediate lobe and at the border between intermediate and posterior lobes. Weak nuclear labeling was found in the pituicytes and generally not in intermediate lobe cells. Varying amounts of radioactivity were found in the colloid in the residual cleft.

In the immunautoradiogram of rat pituitary there was simultaneous localization of peroxidase-labeled anti-hCG and  $^3\text{H}$ -estradiol. Estrogen target cells were characterized by nuclear accumulation of radioactivity (Keefer, Stumpf, Petrusz and Sar—from Stumpf *et al.*, 1975). Heavy cytoplasmic immunostaining was noted in the gonadotrophs. All the immunohistochemically defined gonadotrophs are not estrogen target cells, but most of them are so. Estrogen target cells also included those cells other than gonadotrophs. Cells of the intermediate lobe were unlabeled. These observations were made in ovariectomized rat after injection of  $^3\text{H}$ -estradiol.

The authors concluded that estrogen and androgen concentrated almost exclusively in nuclei. Progestin, glucocorticoid, triiodothyronine, TRH, or their metabolites concentrated in nuclei and cytoplasm of certain cells of the pituitary in varying degrees. The pituitary gland is a hormone target tissue for central neurogenic and peripheral hypophysiotrophic hormones. "The nuclear concentration of hormones corresponds to a nuclear, probably genomic, effect." They further said that as the same tinctorial cell type showed retention and concentration of different hormones, the possibility for an individual cell to be chemically addressed not only by one hormone, but by several hormones, is there. Several cell types may also be addressed by one hormone. Estradiol, triiodothyronine and TRH concentrate in acidophils and all of them stimulate prolactin secretion. A cell type may be transformed into another type by hormone-induction process.

Electron microscopic autoradiography of dispersed pituitary cells of rats was studied by Farquhar, Skutelsky and Hopkins (1975). A new dissociation procedure was described. By autoradiography they assessed the ability of the cell to transport and concentrate secretory products and also determined the intracellular





route which the newly synthesized protein took up. They pulse-labeled the cultured cells as it was shown that the amount of leucine incorporated into STH was twice that found in recently dissociated cells. Only about half of the somatotrophs were heavily labeled and so they were actively engaged in incorporating amino-acids into secretory or other proteins. After 5-min pulse there was a diffuse distribution of most of the autoradiographic grains over the nucleus and cytoplasm of the somatotroph. The striking concentration of grains was over the Golgi apparatus at 15-30 min postpulse. At 60-min postpulse, proportionately less grains were noted over the Golgi and increased numbers were related to secretory granules. At 120-min postpulse the Golgi was free of label and maximum number of grains is related to secretory granules. Newly synthesized, labeled secretory protein is transported from the rough endoplasmic reticulum to the Golgi apparatus where it is packaged into granules. The granules are stored in the cytoplasm.

The authors further concluded that "membrane relocated to the cell surface during exocytosis is recaptured intact and recirculated back to the Golgi. The findings indicate further that the recirculation is restricted to *specific* Golgi cisternae—i.e., the cisternae along the concave Golgi surface—the same cisternae from whence the pieces of membrane originally came."

Pituitary dissociation methods have been used by many and also by Farquhar *et al.* (1975). Hymer (1975) reviewed these studies. With the method used by Farquhar *et al.* (1975) the function and morphological integrity of the cells was preserved and the dispersed system has many advantages over intact tissues. "With dispersed cell suspensions it is possible to: (1) overcome individual variations between glands, since the cell population from a number of glands is randomized, (2) eliminate the diffusion problems encountered in even small tissue fragments, (3) provide rounded-up single cells with a simplified topography (which facilitates certain procedures such as autoradiographic counting), and (4) provide a suitable starting material for the subfractionation of the heterogeneous pituitary cell population into preparations consisting of a single cell type."

Tixier-Vidal (1975) studied the ultrastructure of anterior pituitary cells *in culture*. The study comprised organ cultures, tissue cultures and cell cultures. High level of prolactin secretion and maintenance of the cells for several months *in vitro* reveals a genetic feature of these cells, at least in mammals. Secretory ability of majority of other pituitary hormones decreases with time in culture. Hormonal content of the medium is mainly due to a physiological release of living cells. The gonadotrophic cells undergo morphological modifications and thus a single cell type persists even after two months. TSH, STH and ACTH cells having low autonomy of secretion should also be similarly studied. In mammals prolactin cells and perhaps the melanotrophic cells have maximum level of autonomy. In lower vertebrates TSH cells also have high level of autonomy. Increased autonomy of secretion of several pituitary hormones occurs after malignant transformation. Purified or synthetic hypothalamic hormones and cyclic AMP selectively acts



on the smooth reticulum including the Golgi zone. In culture exocytosis was not particularly evident. Hypothalamic hormones act mainly at the site of segregation and migration of the secretory product. True evidence for transformation of one pituitary cell type into another has not yet been obtained in culture. Cell culture method has limitations because of the overgrowth of fibroblasts. If the starting material is from pituitary tumors then this limitation can be escaped because the dividing ability of the glandular cell now competes with that of the fibroblasts. Sato and his colleagues thus isolated pure pituitary cell lines. "The ultrastructure of such cells, however, is modified with respect to normal prolactin and somatotrophic cells. This fact must be kept in mind in using such cell lines for the study of pituitary cell regulation for which they offer a promising approach."

### *Observations in different mammals :*

Fernandez-Moran and Luft (1949) were the first who studied the anterior pituitary by electron microscope. They used imprinting, smearing and replication methods. Acidophils and basophils could be distinguished.

Cytology of the autografts of rat pituitary in the capsule of the kidney was studied by Rennels (1962). Various cell types could be detected. The largest number of cells had large inclusion granules, dilated cisternae and the Golgi complex contained immature granules. Arrays of endoplasmic reticulum and ribonucleic acid particles were noted. By traumatizing the uterus in the experimental animals, deciduomata could be produced and so the transplants were apparently secreting luteotrophic hormone. The cells possibly correspond to acidophils having large granules and reacting to oestrogen treatment and lactation. As the autografts contain this dominant cell type, it was suggested that these cells secrete luteotrophic hormone.

Green (1966) said, "To date, however, it is still possible to doubt whether thyrotrophs, gonadotrophs, growth hormone acidophils and luteinizing hormone or prolactin secreting acidophils really represent separate strain of cells or merely cells in different stages of activity".

Farquhar (1971) studied the processing of secretory products by cells of the anterior pituitary gland. The anterior lobe of the pituitary produces six hormones : two simple proteins (growth hormone and mammatrophic hormone), three glycoproteins (thyrotrophic, follicle stimulating and luteinizing hormones), and a polypeptide (ACTH). Each hormone is produced by a separate cell type. Nakane (1970) using immunocytochemical tests by peroxidase—labeled antibody technique suggested that a single cell type could produce two gonadotrophins. The secretory granules in different types of cells differed in size, shape and density. The cytoplasmic constituents also showed variations in the distribution and organization. Farquhar stated that the maximal diameters of the secretory granules of



each cell type fell within a characteristic size range (mammotrophs=600-900nm; somatotrophs=350-400nm; gonadotrophs=200-250nm; thyrotrophs=140-200nm; corticotrophs=100-200nm). A typical unit limiting membrane surrounds the granules. Granules of mammotrophs and somatotrophs are more uniformly dense (after  $\text{OsO}_4$  or glutaraldehyde- $\text{OsO}_4$  fixation) than those of thyrotrophs or gonadotrophs. More satisfactory preservation of the granules of the glycoprotein producing cells and corticotrophs is noted by aldehyde followed by  $\text{OsO}_4$  than by  $\text{OsO}_4$  alone. The corticotroph granules appear as *bull's eyes* or target granules with a small dense core (100nm surrounded by a loose-fitting membrane envelope after  $\text{OsO}_4$  alone (Siperstein and Allison, 1965; Kurosumi and Kobayashi, 1966; Yamada and Yamashita, 1967). McShan and his co-workers (McShan and Hartley, 1965; Costoff and McShan, 1969; Hodges and McShan, 1970) substantiated the data of Farquhar and her co-workers by a combination of cell fractionation methods (density gradient centrifugation and filtration). They succeeded in separating the different types of granules and demonstrated the proper biological activities. The secretory granules therefore represent a storage form of the secretory product.

The follicular cells and the stellate cells belong to the *nongranulated* cell class. They are situated mixed with secretory cells in the parenchyma of the anterior pituitary gland. The follicular cells have no secretory granules but they have good number of free polysomes and other cell organelles (Golgi complex, rough ER, mitochondria, lysosomes and lipid droplets). The stellate cell is similar to the follicular cell but has no follicular connection. Previously (Farquhar, 1957) the follicular cells were seen to respond to changes in adrenal function and thus they were thought to represent corticotrophs. Rennels (1964) noted increased number of lipid droplets in follicular cells after stress of scalding. Schechter (1969) proposed a similar function to the stellate cells of the rabbit. At present it is noted that ACTH is produced in a granulated cell type having 100-200nm granules.

Farquhar (1971) made diagrammatic representation of proposed events in the secretory process of mammotrophs in the anterior pituitary of the rat. "MTH is synthesized on ribosomes, segregated and transported by the rough ER and concentrated into granules by Golgi complex. Small granules arising within the inner Golgi cisterna aggregate to comprise the mature secretory granule. During active secretion, the latter fuse with the cell membrane and are discharged into the perivascular spaces by exocytosis. When the secretory activity is suppressed and the cell must dispose of excess stored hormone, some granules fuse with lysosomes and are degraded. This scheme is basically similar to that which takes place in the pancreatic exocrine cell except that (a) concentration begins here in the stacked Golgi cisternae (instead of in specialized condensing vacuoles) and continues in the cytoplasm in something analogous to a condensing vacuole; and (b) there is a discharge option whereby the granules can either be discharged extracellularly (into perivascular spaces) or be disposed of intracellularly by *crinophagy*".



*Stress and ultrastructural changes in the adenohypophysis :*

Pollard *et al.*(1976) obtained good correlation between the cellular activity in the adenohypophysis of the male rat with the circulating levels of corticosterone after prolonged exposure to stress and the investigation comprised morphological and ultrastructural studies. During ten days intense secretory activity was noted in all trophic cells of the adenohypophysis. After this period the cellular morphology returned to normal. Differences in the degree of adaptation were, however, noted amongst the different trophic cells. Hyperactivity in the corticotrophic cells returned to normal level by 20 days. Adaptation to normalcy was noted in thyrotroph activity only after 40 days. Hypertrophic activity in somatotroph and gonadotroph cells returned to control levels by 20 days. After this period an inhibition was observed. Luteotroph activity was exceptionally found to be increased throughout the duration of the stress procedure.

*Control*

*Stress*

- |  |   |
|--|---|
| <p>(1) Control corticotroph is irregular in shape and well granulated. A compact rough endoplasmic reticulum, short rodshaped mitochondria, lysosomes, and a Golgi complex consisting of flattened saccules and vesicles are noted.</p>  | <p>(1) The stressed (1 day) corticotroph cell has an enlarged endoplasmic reticulum with increased number of ribosomes both on the rough endoplasmic reticulum and free in cytoplasm, swollen mitochondria with broken cristae and increased number of lysosomes. 1 and 5 day stressed cells show degranulation, although most cells still have secretory granules present with an extensive Golgi complex. Stellate chromophobe with cytoplasmic projections is also seen.</p> |
| <p>(2) Control FSH-gonadotroph is a relatively large cell, usually round or oval in shape with eccentric nucleus and plenty of secretory granules. Dilated sacs of irregular shape comprise the endoplasmic reticulum. The Golgi complex is well developed, the mitochondria are circular in shape and amorphous bodies unique to the FSH gonadotroph are present.</p> | <p>(2) FSH gonadotroph cell from 1 day stressed pituitary has enlarged and dilated endoplasmic reticulum in which sacs coalesce to form cytoplasmic vacuoles. Increased number of ribosomes, swollen mitochondria with broken cristae, an extensive Golgi apparatus and amorphous body are noted.</p>   |



*Control**Stress*

- (3) The control LH-gonadotroph cell has an endoplasmic reticulum of flattened profiles oriented parallel to the outline of the nucleus and well-granulated cytoplasm. Mitochondria with discontinuous cristae and lysosomes are present.
- (3) 10-day stressed pituitary shows LH-gonadotroph cell with greatly hypertrophied endoplasmic reticulum forming large cytoplasmic vacuoles and lakes which push the rest of the cellular contents and the nucleus to one side. Granules decrease in number and there is an increase in free and attached ribosomes.
- (4) FSH—gonadotroph (60-day stress)—secretory granules are in excess—has electron-dense, tightly packed endoplasmic reticulum and there is an increase in number of amorphous bodies.
- (4) LH-gonadotroph cell (60-day stress) shows secretory granules and an electron-dense endoplasmic reticulum. A large cytoplasmic storage lake is present in the cytoplasm.
- (6) Control luteotroph or prolactin cell has intensive electron-dense and irregular secretory granules with plenty of short cisternae of the endoplasmic reticulum, an extensive Golgi complex and round or short rod-shaped mitochondria.
- (6) Luteotroph cell (10-day stress): endoplasmic reticulum, increased number of ribosomes, swollen mitochondria, several Golgi complexes and granules are noted.
- (7) Storage-somatotroph shows abundant dense-staining granules obscuring most of the endoplasmic reticulum. Mitochondria and a Golgi complex are noted.
- (7) Active somatotroph with secretory granules, a lamellar endoplasmic reticulum at one pole of the cell, an extensive Golgi complex and mitochondria with discontinuous cristae are present.
- (8) Control thyrotroph is a small cell with peripheral arrangement of secretory granules, and poorly developed endoplasmic reticulum and normal looking mitochondria.
- (8) Thyrotroph (20-day stress) is hypertrophic with extensive loss of secretory granules. Lysosomes are increased in number. Mitochondria are swollen and the endoplasmic reticulum is vacuolated.



### *Stress and corticotroph*

Kurosumi (1974) found that stress of laparotomy under ether anaesthesia on rats leads to a marked increase in the total number of secretory granules of corticotrophs after 15 minutes. The granules were still increasing after 30 minutes and they decreased after 60-120 minutes. Increase in the number of granule at 15 and 30 minutes is due to new formation of extremely and moderately dense granules at the Golgi apparatus. At the same time there is a decrease in number of clear vesicles with or without cores. This suggests a release of the content of the granule (ACTH).

### *Mouse and rat :*

Dellmann *et al.* (1973) studied corticotrophic cells in the pars intermedia rostral zone of the *mouse* and *rat* and their relationship to neurohypophysial nerve fibres and the hypophysial portal system. Pars intermedia takes part in the synthesis of ACTH. By rostral zone (RZ) of the pars intermedia (PI) they mean the junctional place of the partes tuberalis, distalis and intermedia. This area has predominance of corticotrophic cells. The stellate cells have secretory granules with mean diameter of about 200nm and they are aligned along the cell membrane. Evidences of secretory stimulation after adrenalectomy in these cells are noted as in the pars distalis corticotrophs. Corticotrophic cellular invasion takes place in the neurohypophysis with perivascular distribution at the transition zone between stalk and posterior lobe. The RZ of the pars intermedia is richly vascularized from the hypophysial portal system. An intimate contact is noted between corticotrophic cells and neurosecretory axons without an interposed basal lamina. These cells also have synaptoid contact with catecholaminergic nerve terminals.

The RZ corticotrophic cells by virtue of their specific localization are influenced by CRF through the capillaries of the superficial plexus and these corticotrophs respond to neurogenic stresses. The central noradrenergic inhibitory system of ACTH secretion can influence the RZ corticotrophs in some way. There is also a possibility of origin of ACTH within the MSH cells of the pars intermedia.

Herlant (1963) confirmed the presence of six types of functional elements in the anterior lobe of the hypophysis by electron microscopy. Differentiation of the cells could be made by the size of their granules and by the structure of their cytoplasmic components, particularly of their ergastoplasm. These criteria mark the distinction between serous and mucoprotein elements. The identification of different types of cells is essentially based on functional relations. Correlation between the functional activity of each of the cellular forms and a state of hypersecretion of the hormone which is attributed to it, could be demonstrated.

Barnes (1963) could demonstrate the cellular origins of five of the six known adenohypophysial hormones—GSH, LTH, FSH, LH, and TSH in the *mouse* by electron microscopic studies of the responses of pituitary cells to changes in physiological state.



Yamada and Yamashita (1967) described the *ACTH cell* in the mouse anterior pituitary. This cell has a relatively large and irregular shape and is situated mainly in the basal part of the gland. The secretory granules have diversified morphology. The round granules which are immature in form are situated in and around the Golgi region. The maximum diameter is 250m $\mu$ . They contain electron dense homogenous substance. At the periphery of the cytoplasm there are many vesicles which often contain a heterogenous material of low density or a dense core. Electron density of the vesicles is gained after double fixation with glutaraldehyde and osmium tetroxide. The rod-shaped mitochondria are aggregated in the Golgi region. Around the mitochondria, the rough surfaced endoplasmic reticulum is located in the form of flattened vesicles. Such cytological features are very similar to those noted in the MSH-producing cells of the pars intermedia of the pituitary. ACTH has a resemblance to MSH in chemical structure. This is an independent cell type in addition to known five others.

After bilateral adrenalectomy the Golgi apparatus is well developed and its vesicles and lamellae are filled with dense material. Many immature granules with dense homogenous substance are prominently present around the Golgi region. At the cell periphery vesiculated mature granules in two or three arrays are found. The mitochondria are aggregated in and around the Golgi region. The endoplasmic reticulum is well developed. In some areas of the cytoplasm, the ER shows flattened cisternae in parallel arrays. Such features are noted at three days after the operation and the maximum change is noted at the fifth day. FSH-producing cell in this circumstance does not show any cytological change.

Typical changes in all FSH-producing cells are noted at 100th day after bilateral orchidectomy. Vacuolar dilatation of the ER takes place. The major part of the cytoplasm is filled with the dilated reticulum. The granules are few and small. The Golgi apparatus is well developed. Large colloid-like dense masses are occasionally found. Under this circumstance there is no noticeable change in the *ACTH cell*. Adrenalectomy following orchidectomy (100-day) stimulates *ACTH cell*. Daily administration of 1 mg of hydrocortisone acetate leads to a hypofunctional state of the *ACTH cell*. The authors conclude that the new cell type identified is an independent cell type and reasonably responsible for the production of ACTH.

Pakurar *et al.* (1975) studied *rat* anterior pituitaries cytologically after cultivation in organ culture and with or without the addition of hypothalamic and cortical extracts. Five distinct cell types could be detected with classical stains in the uncultivated glands; but the peroxidase-labeled antibody technique (using antibodies against STH, LTH, FSH, LH, and TSH) showed that not all of the immune-specific cell types were being identified with the classical stains. Chromophilic cells decreased in number with an increase in culture time. With the peroxidase technique, it was found that all the cells remained constant in type and number, irrespective of time in the culture. Addition of hypothalamic or cortical extracts to the culture medium showed cytological alterations which could be demonstra-



ted by classical dyes, whereas the antibody technique showed no such alterations. This proves that assessment of hormonal activity of the pituitary gland should not be relied only upon histological procedures.

Heap *et al.* (1971) noted the effects of a synthetic antiandrogen cyproterone on the ultrastructure of the *rat* adenohypophysis. Morphological changes in the cells were noted in that part of the pars distalis which abuts on to the pars intermedia. There were degenerating cells. The LH cells indicated active secretion as the secretory granules were aligned against the cell membrane. An LH cell approximating an appearance to a castration cell is also noted. Vacuoles in the cytoplasm of FSH cells were noted rarely. Increased secretory activity in these cells could be judged by the peripheral arrangement of secretory granules. Stimulation of STH cells could also be seen. Macrophages were in abundance. In the central areas of pars distalis there are some apparently non-secreting gonadotrophs.

Shiino and Rennels (1975) found that after surgical thyroidectomy, numerous cytoplasmic microtubules were noted in thyrotrophs, prolactin cells and somatotrophs. Microfilaments were generally plenty in non-granular follicular cells and their relative scarcity were found in most of the secretory cells. Microtubules were noted in the developing thyroidectomy cells. They were noted in contact with or very near the endoplasmic reticula, mitochondria and Golgi components.

The function of the microtubules has been thought to be that they are involved in secretory processes.

Thyroidectomy cells are hyperactive cells originating from thyrotrophs. After thyroidectomy degranulation of thyrotrophs takes place rapidly and thyroidectomy cells appear.

They suggest that microtubules may play a role in degranulation and other processes associated with the hypersecretory state.

### Rabbit :

Foster (1971) studied the relationship between ultrastructure and function in the adenohypophysis of the *rabbit*. Regarding the problem of interpretation of ultrastructural changes it is stated that, "An inevitable consequence is that an increase or suppression of the activity of a particular target organ is likely to affect the ultrastructure not only of the cell species responsible for the production of that particular trophic hormone—the effect is likely to *spread* to other cells. Furthermore, it is not known whether, if at all, changes arising in one type of cell—e.g. castration changes in gonadotrophs—might not, by direct action, exert some effect on neighbouring cells of another type. In practice, it is customary to regard the particular trophic cell as that which shows the most marked changes from the norm".

The pars distalis of the rabbit can be divided into the zona tuberalis (Zt) and the pars distalis proper (pdp). Zt is continuous with the pars tuberalis. There are plenty of PAS-positive mucoid cells and few acidophils near the junction with pdp. In the pdp there are plenty of acidophils with scattered mucoid cells.



The hormone-producing cells of the pars distalis (pdp + zt) are—the prolactin cell, the somatotrophic cell, the gonadotrophic cell, the thyrotrophic cell, and the corticotrophic cell. The non-secreting cells of the adenohypophysis are called *interstitial* cells.

*The prolactin cell :*

They are large, round and acidophilic and more frequently found in the female than in the male. There are large dense round or oval granules, bounded by a membrane. The average size is 420nm (range 250-550nm.). They are situated in pdp. The rough endoplasmic reticulum (ER) is in the form of a few concentric lamellae round the nucleus with scattered elements among the granules of the cytoplasm. Striking cellular changes are noted in late pregnancy and lactation. Increase in ER occurs and many cells are packed with large dense granules. During early lactation extensive degranulation with a greatly increased ergastoplasm and a very prominent Golgi area develops.

*The somatotrophic cell :*

Situation	— pdp and very occasionally in the zt.
Size	— Small
Shape	— as that of prolactin cells
Dense granules	} — 270nm (average size)
Form and distribution of the ergastoplasm and relatively few mitochondria	} — Same as noted in prolactin cells

*The gonadotrophic cell :*

Separation between cells producing FSH and LH could not be done convincingly. By light microscope the authors could state that LH—gonadotrophs were predominantly in pdp and FSH—gonadotrophs in zt. Gonadotrophs in zt. contain larger granules than those in pdp.

The shape of these cells is irregular. The ER is in the form of lamellae and they are concentrically arranged around the nucleus. The granules are smaller and less dense compared to those of somatotrophs. The average granular size in zt is 220nm and that in pdp is 150nm. The mitochondria are concentrated at one pole. Following castration there is an extensive degranulation and hypertrophy of the Golgi zone and dilatation of the cisternae of the ER. After a long time spherical dilatation filled with homogeneous but not very dense material is noted. They remain discrete but occasionally coalescence has been noted.

*The thyrotrophic cell :*

This small cell is more irregular in shape than the gonadotroph. There are long processes in between other cells. The average size of the granule is 100nm



in pdp and 120nm in zt. These granules are often seen aligned against the plasma membrane. The ER is not plenty. The mitochondria are few. Propylthiouracil leads to partial suppression of thyroxine production with an increased production of TSH and resulting in a greatly hypertrophied thyroid. Changes start in gonadotrophs, somatotrophs and presumed thyrotrophs. There is degranulation response in thyrotrophs. The ER increases in amount with parallel arrays of lamellae and cisternal dilatations. These are irregular in shape and size as opposed to those noted in castration cells.

#### *The corticotrophic cell :*

The cellular source of ACTH is controversial. The cell is difficult to identify in normal rabbits. After injections of metyrapone for various periods these cells are frequently found in pdp and zt. The corticotroph is irregular like thyrotroph and the granules are membrane-bound. The average diameter is 170nm. A clear space is noted in between the granules and the membrane (*halo cells of Dingemans*). The stretch of the ER is longer than that noted in the thyrotroph. It is sinuous and snake-like with occasional branches and anastomoses. There are free ribosomes. The Golgi zone is concentrated with small vesicles, vacuoles and flattened cisternae.

#### *The interstitial cells :*

They are located in the pars distalis, the pars intermedia and the pars tuberalis and are similar to *stellate* and *follicular* cells. Phagocytic and absorptive activity has been ascribed to them. "The *interstitial* system may be concerned with the maintenance of a circulation of intracellular fluid by something resembling a pseudopodial deformation of the cell surfaces". These cells could function as a motive force in transfer of hormones to capillaries. Their action may be supportive or sustentacular. Schechter(1969) noted an activation of interstitial cells after treatment with metyrapone. The Golgi area is enlarged and there is an increased density of microfibrils. These cells may produce ACTH according to Schechter.

#### *Mink*

Murphy and James(1976) studied the cells of the adenohypophysis of the mink(*Mustela vison*) identified by immunohistochemical and functional criteria. Six cell types have been known to exist in the anterior hypophysis of mammals and they produce at least six types of hormones. "One cell, one hormone" theory has been accepted by most authors; but for the rat Nakane (1970, 1971) suggested that gonadotrophic cells produce FSH & LH. Ultrastructural identification of the pituitary cell types was done by immunolocalization in the rat by Nakane(1971), Mazurkiewicz and Nakane(1972), Tougard *et al.*(1973) and Parsons and Erlandsen(1974).

Ultrastructural identification of different cell types in the adenohypophysis of the dog was done by Kagayama(1965) and Gale(1972).



*Gonadotrophic cells in the adenohypophysis of the mink :*

The authors designated gonadotrophic cells as those which were immunoreactive to antisera to LH and HCG.

Pleomorphic cells immunoreactive at the light  
microscopic level.

Type I

Type II

Shape : Oblong

Nucleus : Centrally located

Cytoplasm : Vacuolated

AF } after  
AT }  $\text{KMnO}_4$       +  
         oxidation

Fast Green      -

Aniline blue      +

Acid fuchsin      +

PAS }  
OG }

Large

Centrally located

Homogeneous

+

+

+

+

+

FSH cell

LH cell

*EM level*

Type I

Type II

Immunoreaction  
with antisera  
to LH :

Immuno-  
reactive

Immuno-reactive

Hormone  
granules :

Spherical,  
uniform, 300nm  
in diameter

Dense, 150 to 300nm in diameter

Nucleus :

Central &  
often invaginated

Round, eccentric invaginations  
noted

Cytoplasm :

Slightly electron-dense,  
vacuolated

clear, rarely vacuolated



	Type I	Type II
Mitochondria :	Round and sometimes oblong	Pleomorphic
Location of cells :		Located at sinusoidal lumina & in clusters
Corresponds to :	LH cell of Mikami, Rat FSH type A cell of Nakane, and FSH cells of Herlant	LH cells of Nakane, gonadotrophic basophil cells of Lever and Peterson, FSH cell of Nakayama <i>et al.</i> , gonadotrophs of Rennels <i>et al.</i> , and LH cells of Herlant.
Castration :	Partial degranulation, increase in electron density of cytoplasm, secretory granules coalesce, and vacuolation of cytoplasm	Complete degranulation, vacuolation of cisternae of endoplasmic reticulum. In some cells cytoplasm is extremely vacuolated and nuclei are frequently invaginated.

#### *Thyrotrophic cells :*

These cells contain small, uniform hormone granules (100—150nm). Many of these granules have a dense core surrounded by a perigranular halo, the packaging membrane.

After thyroidectomy the cells hypertrophy in the mink and they contain only small granules. Thyroidectomy cells have large and small saccular dilatations in the cytoplasm. Many of these vacuoles are studded with ribosomes and this indicates that they are swollen rough endoplasmic reticulum. There is dispersion of nuclear chromatin with a prominent nucleolus. Hypertrophied Golgi and elongated mitochondria are noted.

#### *Acidophils :*

LM — Single acidophilic cell type. OG, acid fuchsin, and azocarmine positive. Located at central area and cords of such cells proceed towards the periphery of the gland.

#### *EM — two types of cells :*

	Type I Acidophil	Type II Acidophil
	STH cell	Cell producing PRL
Secretory granules :	Dense, uniform. Absence of perigranular membrane	Large, very dense, irregular



Nucleus :	Central	dispersed chromatin
Golgi apparatus :	Inconspicuous	
Intranuclear invagination of cytoplasm :		present

## Type I

Type II  
Mammotroph

2 days after loss or removal of suckling young :

- (1) Involution of degranulated cells show increased electron density of the cytoplasm. Golgi apparatus functional. Focal enlargement of the perinuclear cisterna. Expansion of Golgi saccules.
- (2) Involution of granulated mammothrophs—granular dissolution.

*The amphophil or ACTH cell :*

LM : These cells are weakly PAS positive and have slight affinity for basophilic dyes e.g. aniline blue and haematoxylin. They may be detected singly or they may form a follicle surrounding a colloid-like center and are located in the central portion of the gland.

EM : ACTH granules are small in size (150nm) and they are peripherally arranged. Glycogenic rosette formations are common.

The colloid in the follicles is bounded by the plasma membranes of the ACTH cells. The nuclei are basal in location. The presence of microvilli on adjacent ACTH cells suggests that the function of the colloid may be storage of polypeptide hormone or carrier protein.

*Long-term responses to adrenalectomy :*

- Relatively complete degranulation of ACTH cells with persistence of immature granules is noted. There is resorption of the colloid and formation of large symmetrical vacuoles in the cytoplasm. Many of the vacuoles have an inner rim of colloid-like material.
- Cytoplasm is filled with electron-dense fragments symmetrically aggregated. The hypertrophy of the ACTH cells after adrenalectomy is so much as to produce the largest cells of the pituitary.

*Monkey :*

Costoff(1977) studied the ultrastructure of the pituitary gland of Rhesus monkeys. The pars tuberalis cells are similar to those of pars distalis but they are smaller in size and the secretory granules are 200 to 500nm in diameter. The primate anterior pituitary gland produces at least six hormones.



Cells secreting protein hormones are STH secreting cells, prolactin secreting cells and corticotrophs and the distinguishing stains for them are orange G, erythrosin and tetrachrome respectively with maximum diameters(nm) for the secretory granules being 350-500, 600-1000, 150-200 respectively. The glycoprotein hormone secreting cells are TSH cells and gonadotrophs (FSH and LH cells) and the distinguishing stain for them are aldehyde fuchsin and PAS respectively with the maximum diameters(nm) for the secretory granules being 100-150 and 200-300 respectively. Follicle cells and chromophobes do not produce hormones.

At least 50% of the cells of the monkey adenohypophysis are somatotrophs. The shape of this type of cell is ovoid and there is one well developed Golgi complex. RER is characterized by short cisternae. Frequently a centriole with a single cilium and lysosomes have been observed.

The prolactin cells are plenty in female monkeys and this is the dominant cell type during pregnancy and in renal autografts of the pituitary. These cells were also found in foetal pituitaries near term. They have *largest secretory granules* which are ovoid or irregular in shape. Immature granules in different stages of production have been noted in the Golgi complex. Extensive endoplasmic reticulum has been found during late pregnancy or lactation (active cells). They have also been found in male monkeys and the secretory granules are plenty in castrated male and female monkeys. In the renal autografted pituitary, prolactin was actively synthesized, stored and secreted by these cells.

Corticotrophs are few in number. The shape is either angular or stellate. The endoplasmic reticulum is of the scattered vesicular type or short lamellar type. The Golgi complex is small. Lysosome can also be seen.

Thyrotrophs are *least abundant* in the monkey pituitary and they have *secretory granules of smallest diameter*. The cells are small and angular and are found in groups. The secretory granules are distributed at the periphery of the cell membrane.

Gonadotrophs have pleomorphic nuclei and the Golgi complex is well developed depending on the physiological state. Before the ovulatory surge of gonadotrophins these cells have extensive endoplasmic reticulum. After Oophorectomy the gonadotroph is greatly enlarged, the secretory granules are plenty in number, and endoplasmic reticulum is vacuolated. Golgi complexes are there. The mitochondria are swollen. The signet ring-like cell (typical castration cell) is found six months after oophorectomy. The endoplasmic reticulum is enlarged and vacuolated. In long term (1 yr) castrates the gonadotrophs are vacuolated and the secretory granules are few. Immunocytochemical studies in man (Phifer *et al.*, 1973) and in the rat (Moriarty, 1975) reveal that FSH and LH are present in the same cell on many occasions, though ultrastructurally the two types of gonadotrophs are separate.

The chromophobes contain little cytoplasm and few cellular organelles.  
*Immunocytological observations:*



Fontaine and Olivereau (1975) pointed out that in mammals cell types based on staining reactions do not always agree with the results of immunofluorescence studies. Moriarty (1973) found that corticotrophic cells in several mammalian species are intensely PAS positive. Dubois (1971) noted that in cattle, sheep and pigs cells previously found to secrete LH, now have been found to secrete ACTH.  $\beta$ -MSH is also secreted by these cells. In the pars intermedia there is ACTH or a peptide (corticotrophin-like intermediate lobe peptide, CLIP) with corticotrophic activity. Its structure is very similar to the 18-39 amino acid sequence of ACTH. Fontaine and Olivereau (1975) said that this "also poses an interesting problem, specially as there is cytological evidence that ACTH is secreted by the intermediate lobe of the rat (Porte *et al.*, 1971; Moriarty and Halmi, 1972; Stoeckel *et al.*, 1973). The possibility that ACTH in the intermediate lobe is a precursor of MSH (which has the 1-13 sequence of ACTH), with CLIP as a cleavage product of the parent molecule, has been envisaged".

Another example has been cited by Fontaine and Olivereau (1975). Gonadotrophin production in mammals occurs in two distinct cell types. The homology between the two glycoprotein hormone groups (gonadotrophins and thyrotrophin) (Fontaine, 1967) has recently been confirmed by the demonstration that LH and TSH possess  $\alpha$  and  $\beta$  subunits. In both the hormones  $\alpha$  subunit is common (Pierce, 1971; Papkoff, 1972). This explains to certain extent the apparently contradictory histological findings.

Immunocytochemical studies show that in the rat the  $\alpha$  and  $\beta$  subunits of LH are located in the same gonadotrophic cells which are PAS and AB positive and in the majority of TSH cells which are AB positive. The two LH subunits are always found in the gonadotrophic cells whereas the TSH cells have the non-specific  $\alpha$  subunit; they contain both subunits rarely but never the  $\beta$  subunit only. Gonadotrophic cells and some TSH cells contain LH (Tougaard *et al.*, 1972). Nakane (1970) found immunochemically that FSH and LH are located in the same cells in the rat.

#### *The histology and pathology of the pituitary gland*

Warner (1977) discussed the histology and pathology of the pituitary gland in volume two of *Pathology* edited by Anderson and Kissane. Three types of epithelial cells can be identified by haematoxylin-eosin staining in the pars distalis. Chromophil cells with acidophilic granules are about 40%, chromophil cells with basophilic granules are about 10%, and chromophobe cells with no visible granules are about 50%. The functional classification of cell types has been widely accepted now. STH and LTH are simple proteins. FSH, LH and TSH are mucoproteins. ACTH and MSH are polypeptides. McManus (1946) first noted PAS + granules in pituitary cells and Pearse (1952) studied the cytochemistry and cytology of the normal anterior hypophysis by the trichrome-PAS method and pituitary basophils could be identified. With PAS-orange G method,



acidophils are orange G +. Basophils containing mucoprotein hormones (FSH, LH, and TSH) are PAS + and are called mucoid cells. Chromophobes have no visible granules. Acidophils have affinity for eosin and other acid dyes e.g. orange G, erythrosin and carmoisine (Brookes, 1968). STH cells and LTH cells are acidophils and in horizontal section of the pituitary they are situated in the lateral wings. By Herlant's tetrachrome method somatotrophs are orange G + and LTH cells (lactotrophs) are erythrosin +. By electron microscopy there are plenty of granules in the somatotrophs and the granule diameter is 350 nm. Sparse granules have been noted in the LTH cells. These granules measure upto 750 nm and the rough endoplasmic reticulum is most abundant when compared to other cell types. The LTH cells also contain concentric whorls of rough endoplasmic reticulum which are known as *nebenkern* and this is a very prominent feature. During pregnancy the lactotrophs are greatly enlarged and are called *pregnancy cells of Erdheim* and this can be easily differentiated from the somatotrophs even by haematoxylin and eosin stain by their large size. Gonadotrophs (FSH cells and LH cells), thyrotrophs and corticomelanotrophs (producing ACTH and MSH) are basophils. FSH, LH and TSH containing cells are mucoid cells containing glycoprotein (protein + polysaccharide). Free aldehyde groups are formed in the polysaccharide after oxidation with periodic acid and Schiff's reagent demonstrates these aldehyde groups by formation of purple complexes. The mucoid cells could be further separated by Adams and Sweetenham (1958). Two types of granules were described : S (susceptible) and R (resistant). They used preliminary oxidation by performic acid, followed by alcian blue, PAS and orange G (PFA-AB-PAS-orange G). With extraction by performic acid, the R granules are resistant and they stain red with Schiff's reagent. S granules contain very high level of cystine and they are susceptible to extraction with performic acid. They stain not with PAS but with alcian blue. The R cells are corticomelanotrophs and they contain red granules. Pearse and van Noorden (1963) further divided the S cells into blue S<sub>1</sub> (gonadotrophs) and purple S<sub>2</sub> cells (thyrotrophs). With PFA-AB-PAS-orange G, the acidophils are orange G+. Chromophobes have no visible granules.

With permanganate-aldehydethionin-PAS-orange G stain (Ezrin and Murray), the acidophils are orange G+, the gonadotrophs are PAS+ (magenta granules) and the shape of the cell is round. The thyrotroph is thionin+ with blue purple granules and the shape of the cell is angular. Corticomelanotrophs are PAS+ with red granules and the shape of the cell is oval. There are no visible granules in chromophobes.

In horizontal sections the basophils are plenty in number in the posterior part of the median wedge. Phifer, Midgley and Spicer (1973) found by immunohistologic and histologic evidences that follicle-stimulating hormone and luteinizing hormone are present in the same cell type in the human pars distalis and so the cell is called as FSH-LH cell. Ultrastructurally the gonadotrophs have granules of 200 to 250 nm in diameter (fig. 6.15). In the human pituitary von Lawzewitsch, Dickmann, Amezua and Pardal (1972) identified two types of



gonadotrophs by cytological and ultrastructural studies. The thyrotrophs in horizontal section are located in the anterior and subcapsular region of the median wedge. Ultrastructurally the dense granules of thyrotrophs are 150 to 200nm in diameter and they are distributed peripherally. The shape of the cells are elongated. These cells can be immunohistologically stained. The shapes of the corticomelanotrophs or ACTH-MSH cells are oval and they are large cells. In horizontal section they are more concentrated in the anterior median wedge and nearby lateral wings. Another group of ACTH-MSH cells are found to infiltrate the pars nervosa, very near the pars distalis. MSH is found in two forms:  $\alpha$ -MSH and  $\beta$ -MSH.  $\beta$ -MSH is the main hormone in man. Amino acid sequences are same in  $\alpha$ -MSH,  $\beta$ -MSH and ACTH and thus there is immunologic cross reactivity. Phifer, Orth, and Spicer (1974) specifically demonstrated ACTH-MSH cell in the human hypophysis and the cell contain both  $\alpha$ -MSH and  $\beta$ -MSH over and above ACTH content. They used histochemical techniques and immunostaining of serial sections. The granules of the cell measure 100 to 200nm in diameter and filaments have been noted in the cytoplasm. Chromophobes may be primordial or resting cells. A group of chromophobes can be ultrastructurally found to consist of granules of 150nm or less in diameter and they secrete ACTH. Another group of chromophobes with very fine granules does not secrete any known hormone. The third group of chromophobes are follicular or stellate cells. These cells have microvilli, cilia, junctional complexes at the follicular pole, stacking of organelles and elongated mitochondria. Their functions have already been described.

*Two distinct types of microfilaments in the cytoplasm of human adenohypophyseal cells:*

Horvath *et al.* (1975) found two distinct types of microfilaments in the cytoplasm of human adenohypophyseal cells and the materials for study consisted of 5 pituitary glands removed from breast cancer patients treated with glucocorticoids, 3 glands obtained from diabetic subjects with vascular complications including retinopathy, and 18 pituitary adenomas. Nine out of 18 pituitary adenoma cases had acromegaly with clinical and laboratory signs of increased growth hormone production. Eight subjects had *chromophobe* adenoma with headache, visual disturbances and mild to moderate hypopituitarism. The other patient had a pituitary tumor with unusual clinical features.

*Type I microfilaments:* These microfilaments were found in pituitaries of five glucocorticoid-treated breast cancer patients (Crooke's cells), in some adenohypophyseal cells of three diabetic subjects and in a pituitary adenoma showing acidophilia and PAS-positivity by light microscopy. The type I microfilaments were of 70Å in width and they formed parallel bundles either in the perikaryon or scattered throughout the cytoplasm. They were frequently found in large cytoplasmic areas in Crooke's cells and in pituitary adenoma cells. The type I microfilaments were consistently associated with free ribosomes. The authors found microfilaments also in cells which did not



show the histological characteristics of Crooke's cells. Type I microfilaments interfere with the discharge of secretory material either by promoting or inhibiting it.

*Type II microfilaments:* These microfilaments were found in the cells of five out of nine acidophilic adenomas with acromegaly. The average width of the filaments was 115Å. They were randomly oriented forming circular bundles and thus building up fairly large spherical intracytoplasmic bodies. The filaments had spatial relationship to tubular smoothsurfaced endoplasmic reticulum and Golgi zones. The spherical bodies are indicators of some metabolic derangement which may or may not be connected with secretory activity.

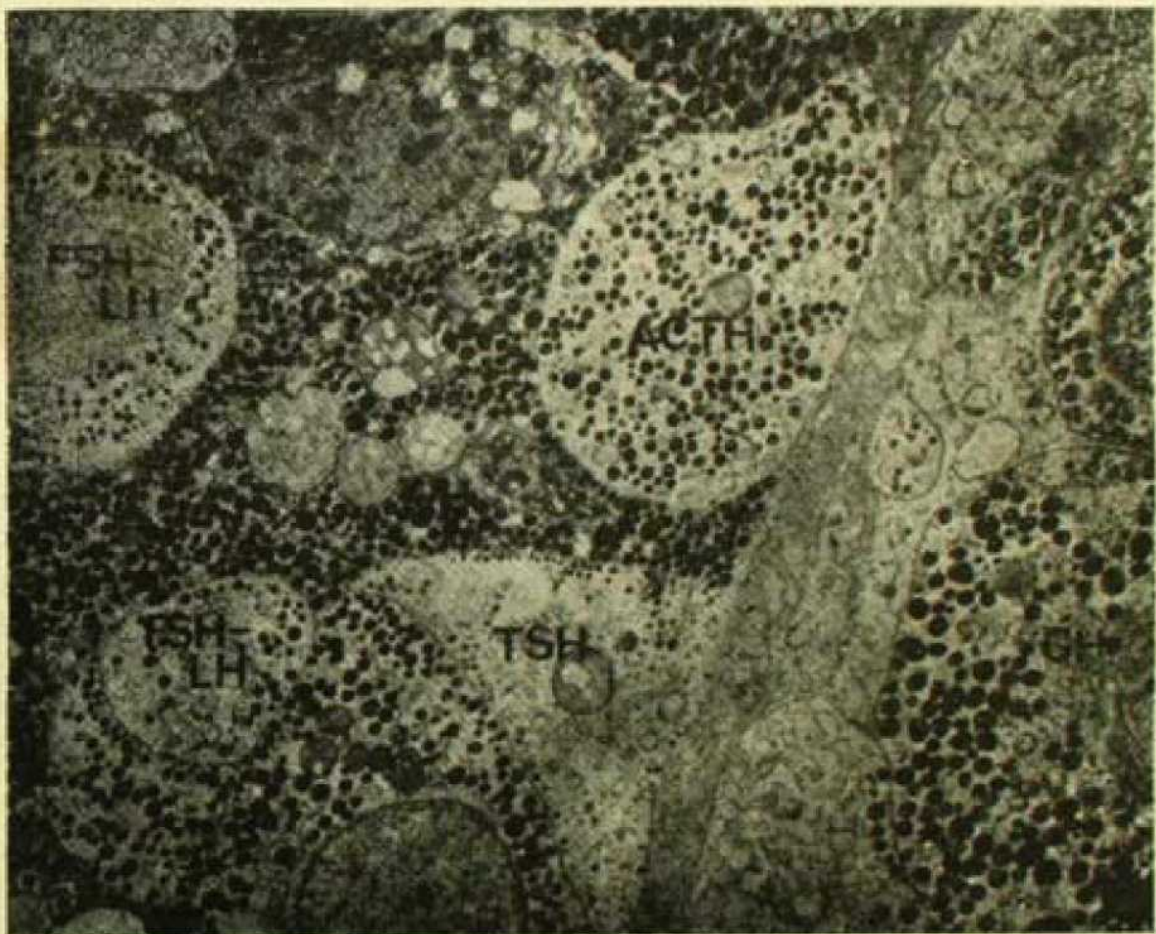


Fig. 6.15. Electronmicrograph  $\times 4490$  of normal pituitary removed trans-sphenoidally from a woman aged 56 with advanced carcinoma of breast. The cell with the smallest granules is a presumed thyrotroph (TSH) and the largest granules a presumed growth hormone (GH) cell. Slightly smaller granules are present in the presumed ACTH cells. The presumed FSH-LH cells contain granules larger than TSH and smaller than ACTH. The actual measurements of mean granule size in nm in this electronmicrograph are : TSH 140, FSH-LH 230, ACTH 330, GH 470 (Gray, 1976). Courtesy of Professor Doniach and W. B. Saunders Co. Ltd. London, Philadelphia, Toronto.

#### *Adrenocortical hyperfunction*

This was discussed by Currie (1971) and Warner (1977). In adrenal hyperactivity of Cushing's syndrome Crooke's hyaline cells are met with in the hypophysis. This change is also found in cases with increased adrenocortical





hormones in the blood. Increased adrenocortical hormones in circulation is also met with after administration of exogenous glucocorticoids, and hypercorticism developed due to ectopic production of ACTH in lung cancer. The affected basophilic cells are the ACTH-MSH cells or corticomelanotrophs. There is accumulation of the hyaline material first around the nucleus and subsequently it spreads throughout the cytoplasm.

Ultrastructurally Crooke's cell has very fine filaments in the hyaline cytoplasm embracing secretory granules, ribosomes and lysosomes. With immunofluorescence studies it has been found that corticotrophin is present in the granular parts of Crooke's cells and is absent from the hyaline portions.

#### *Ultrastructure of human pituitary tumors*

Ultrastructural studies have been conducted by Schelin(1962), Gusek(1962), Fukumitsu(1964), Porcile and Racadot(1966), Cardell and Knighton(1966), Kuromatsu(1968), Bergland and Torack(1969) and Paiz and Henniger(1970).

Schechter(1973) studied ten cases of human pituitary tumors (chromophobic adenoma) by electron microscopy. Hypopituitarism was found in seven and acromegaly in three cases. There was slight morphological variation in the parenchymal cell types when the tumors were compared and a predominant single type was common to both varieties of the tumor based on characteristics of the secretory granules. The predominant cell type had varying amounts of secretory granules (80-200m $\mu$  or more in diameter) and these granules had an electronopaque core and perigranular halo. The author thought that though the features of these secretory granules were most like those in *normal* adult basophils, this classification was considered doubtful. Some granules had electronopaque core material while in others it was particulate. Many secretory granules contained also aggregates of clear vesicles. Some granules do not contain clear vesicles but they were closely associated with plenty of cytoplasmic vesicles. The tumor cells had abundant lysosomes. Ultrastructural criteria used to identify cell types in normal pituitary glands are not applicable to the tumor cells (fig. 6.16).

Chromophobe adenoma with acromegaly varies more with respect to the features of the predominant cell type than was noted in chromophobe adenoma associated with hypopituitarism. On the one hand cells with electronlucent cytoplasmic matrix and scanty development of organelles were found, while on the other there were cells with more electronopaque cytoplasmic matrix and more complex development of organelles. Number and structure of mitochondria varied from cell to cell. Sometimes they filled the cytoplasm.

In the same year Schechter(1973) studied nine cases of acidophilic adenoma associated with acromegaly by electron microscopy. The tumor contained plenty of somatotrophs. Many of them were *normal* and engaged in protein synthesis. Some somatotrophs had a marked electron opacity of the cytoplasmic matrix



and nuclear materials, irregular cell outline, hypertrophied cytomembrane systems frequently having proteinaceous material, and considerable amounts of secretory granules.

Plasma membranes were disrupted throughout the parenchyma and specially at the parenchymal-pericapillary interface. Through these disruptions large numbers of secretory granules and cytoplasmic fragments were released into the pericapillary or extracellular spaces. Small groups of disrupted cells often were characterized by scanty secretory granules, an electronlucent cytoplasmic matrix, and these were considered by the author as the counterpart of giant multinucleate chromophobes as seen by light microscope.

The follicular cells were rarely observed. These cells had abundant fine cytoplasmic filaments and closely resembled follicular cells described in ACTH secreting tumors.

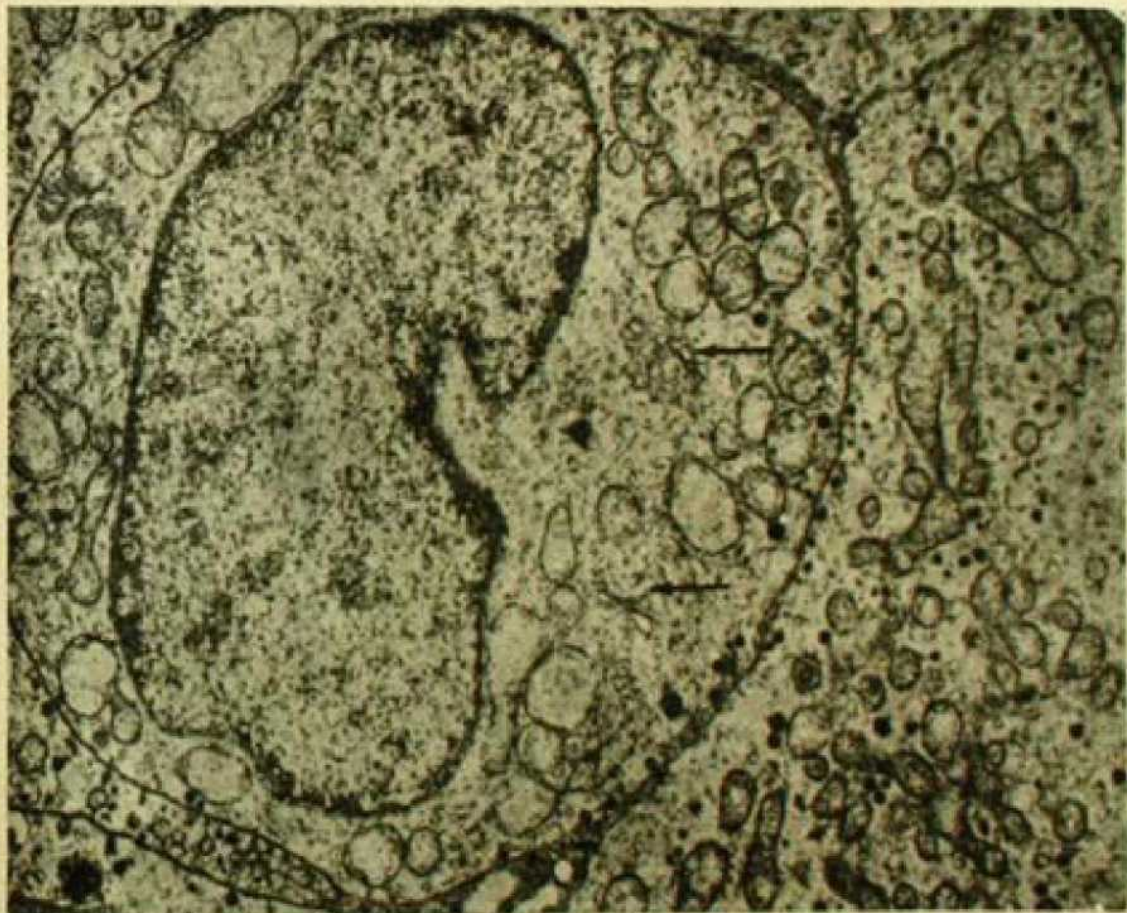


Fig. 6.16. Electronmicrograph  $\times 10000$  of a clinically non-functioning chromophobe adenoma removed from a woman of 56. Note the presence of sparse secretory granules (mean diameter 150 nm), mitochondria and Golgi apparatus (arrowed), (Gray).  
Courtesy of Professor Doniach (1977) and W. B. Saunders Company Ltd. London. Philadelphia, Toronto.

Tumors involving somatotrophs produce the syndrome of gigantism/acromegaly: lactotrophs produce amenorrhea-galactorrhea syndrome: thyrotrophs produce hyperthyroidism: tumors of cortico-melanotrophs produce Cushing's



syndrome/Nelson's syndrome : chromophobe tumors produce local compression effects, impaired vision and hypopituitarism (Warner, 1977).

Olivier, Vila-Porcile, Racadot, Peillon and Racadot (1975) described the ultra-structure of pituitary tumor cells with a review on the subject. Previously it was known that in hyperpituitarism there was excess secretion of either STH or ACTH-MSH or LTH or TSH but at present it has been found that two hormones can be secreted at a time : STH + LTH or STH + ACTH or LTH + ACTH or STH + TSH.

Olivier *et al.* (1975) studied 96 cases of acromegaly (of which 24 by electron microscopy), 25 cases of amenorrhea-galactorrhoea (12 by electron microscopy), 25 cases of Cushing's disease (16 by electron microscopy) and 150 cases of chromophobe adenomas (120 by electron microscopy).

#### *Experimental pituitary tumors :*

There is excellent description of these tumors by Furth and Clifton (1966) and by Olivier *et al.* (1975). Experimental pituitary tumors can be formed by induction and transplantation of spontaneous or induced tumors. Induction is caused by different specific sustained homeostatic derangements, by carcinogens or by a tumor induction by radiothyroidectomy in mice producing thyrotroph tumors and estrogen treatment in mice and rats producing mammosomatotroph tumors.

#### *Thyrotroph tumors*

These tumors can be induced by radiothyroidectomy, continued administration of propylthiouracil and surgical thyroidectomy. All these procedures lead to deficiency of thyroid hormone. In rats partial thyroidectomy leads to pituitary tumor.

After radiothyroidectomy the thyrotrophs are converted into thyroidectomy cells by degranulation, hyperplasia and hypertrophy. Focal adenomas composed of thyroidectomy cells are found after about six months. After about ten months gross tumors are formed by similar but less differentiated cells. The thyrotroph tumor cells may be amphophilic or they are chromophobe cell adenomas. The chromophobe appearance may be due to discharge of the hormone carrying granules. They may also be undifferentiated precursor cells. The chromophobe cells accumulate granules after thyroxine administration because thyroxine blocks the release of granules and so neoformation of the granules takes place. Ultrastructurally the tumor cells were large with plenty of vesicles of different sizes. The Golgi apparatus was prominent and few secretory granules were observed.

In the stage of microadenoma formation there are chromophobe cells with little cytoplasm and without granules. Necrosis is frequently met with.



In the gross tumor stage, whole pituitary organization disappears and the original parenchyma is scanty. Haemorrhagic and necrotic areas are common. The tumor cells are chromophobes with mitosis.

The granules in the transplanted tumors are of 30 to 80nm in diameter or of 150nm in diameter.

In every stage of the tumor formation there is evidence that it is composed of thyroidectomy cells. They may be modified thyrotrophs, or chromophobes of thyrotrophic nature or even may be due to transformation of gonadotrophs, somatotrophs or of cells with *haloed secretory granules*.

Thyrotroph tumors may also have gonadotroph functional activity in some mutant autonomous variants. Two-hormone formations were demonstrated by immunocytochemistry. Similarly thyrotroph tumors can have somatotroph activity (proved by immunocytochemistry). Thyrotroph autonomous tumor may become mammosomatotroph.

#### *Adrenotrophic tumors*

These tumors are induced in mice by direct irradiation of the pituitary gland. Spontaneous tumors have not been reported in laboratory animals. The adrenotrophic tumor cells are smaller than tumor cells of thyrotrophs and mammosomatotrophs. They have sparse to moderate amounts of cytoplasm having very few cytoplasmic vesicles, relatively few mitochondria, Golgi elements and endoplasmic reticulum. These cells closely resemble those of pars intermedia. Tumor growth was accelerated by adrenalectomy. Hypertrophy of the zonae fasciculata and reticularis of the host adrenal cortex has been noted. No change has been encountered in the zona glomerulosa. Atrophy of the X zone is found. There is obesity, lymphopenia, eosinopenia, adrenal hypertrophy, polyuria, polydipsia, hypernatraemia, leucopenia, ovarian and thymic atrophy, cessation of the oestrus cycle and susceptibility to infection. These are associated with typical fatal Cushing's syndrome caused by grafted adrenotrophic tumors. In tumor-bearing mice increased level of corticosteroids and ketosteroids in blood has been found. The corticotrophic hormone content of the tumor was found to be increased.

#### *Mammotroph and mammosomatotrophic tumors*

These tumors are induced by prolonged treatment with massive doses of natural or synthetic estrogens. These are tumors of acidophilic cells. Promotion of growth and mammary gland stimulation have been noted.

Firstly there is hyperplasia followed by adenoma and finally tumor formation. After serial transplantation, autonomous variants arise which are capable of tumor formation in unconditioned hosts. Although capability of growth in gonadectomized animals is there but most autonomous mammosomatotrophic tumor retains responsiveness to estrogen. Estrogen-induced pituitary tumor was



previously thought to be chromophobic but subsequently it was found to arise from acidophils. With estrogen treatment these cells hypertrophy, degranulate and proliferate.

Ultrastructurally these cells are large and contain dense, ovoid or globular cytoplasmic granules. The mitochondria are pale and there are plenty of endoplasmic reticulum from tiny ovoid profiles to parallel arrays of cisternae which are characteristics of normal acidophils (Farquhar and Furth, 1959). The modifications of the mammotrophs include hypertrophy of the ergastoplasm with a lamellar and whorled aspect of the cisternae producing *Nebenkern* pictures. There is hypertrophy of Golgi apparatus and the nucleolus is large. Exocytosis is quite common. Mitosis has been found in granular (mammotrophs) and nongranular cells.

Several months after estrogen treatment tumor formation happens and many haemorrhagic areas can be seen. Other cell types e.g. thyrotrophs, and corticotrophs are active. Other unidentifiable (chromophobe) cells are found. Some tumor may show mammotroph, somatotroph and corticotroph. MtT/F4 strain is functionally mammosomatocorticotroph but cytologically *chromophobic* under light microscope. Ultrastructurally the cells do not show any characteristic of the pituitary cell. There is reduction of ergastoplasm and Golgi apparatus, free ribosomes are plenty, and secretory granules are scanty or absent. The diameter of the granules varies between 150 and 220nm or it is 350nm. The three hormones may be secreted by one cell or by several cells of the MtT/F4 tumor.

A clonal strain from MtT/W5 secretes somatotrophic and mammotrophic hormones. Ultrastructurally the cultures consisted of poorly granulated cells (150 to 250nm). The secretion of these cells could be modified by hydrocortisone or TRF. With TRF there is hypertrophy of Golgi area with increase in the number of granules.

Corticotrophic activity has also been noted in mammosomatotrophic tumor. Adrenocortical hypertrophy with vacuolar degeneration starting from zona reticularis and proceeding outwards has been noted. Terminal cortical haemorrhage is also met with. Increase in plasma corticosteroid level in adrenal venous effluent was noted in MtT/F4 rats. These tumors grafted in the hindleg of rats could be stimulated by minute amounts of vasopressin injected into the artery supplying the tumor resulting in the increase of corticotrophin.

#### *Mammotrophs of estrogen-induced pituitary tumor :*

Induction of hyperplasia or tumor occurs in the anterior pituitary gland by continuous estrogen therapy to female rats. This tumor was called chromophobe adenoma because the cells did not take up any stain, when viewed under light microscope but ultrastructurally they had well developed endoplasmic reticulum and there were few secretory granules which marked these cells as lactotrophs (mammotrophs). Estrogen either administered for a short or a long time lead to the hypertrophy of mammotrophs and this may be due to either its action



on the pituitary cells or its action through the hypothalamus. Estrogen injected into the hypothalamic artery leads to the inhibition of the production of prolactin-inhibiting hormone and thus it stimulates the mammotrophs. There is hypertrophy of these cells with extensive production of prolactin. Increased development of the acini of the mammary gland in such rats speaks in favour of increased prolactin level in the blood. Upset in the lipid metabolism of these mammotrophs (Watari and Tsukagoshi, 1969) leads to the accumulation of round or stellate shaped lipid droplets. These were not thought to be evidences of degeneration however. Rather there were some dark cells amidst the hypertrophied mammotrophs which might be degenerated mammotrophs or some other cell types. These features are observed after three or more months of estrogen treatment.

Kurosumi (1974) stated that severe degeneration could be found in some cells of the pituitary tumor after prolonged administration of estrogen. Watari and Tsukagoshi (1969) noted the absence of ribosomes from many parts of the rough-surfaced endoplasmic reticulum with formation of smooth-surfaced membranes at places. They explained this finding by two possibilities. Ribosomes may be detached from the rough-surfaced endoplasmic reticulum. The other possibility is that "there is new formation of smooth membrane in the stack of rough ER". Membranes in the ER multiply without increase in the number of ribosomes. Kurosumi (1974) stated. "The distance between two opposing outer (matrical) surfaces of ER membranes is smaller in between the smooth parts than in between the rough (granular) parts."

In the central part of the whorl-like lamellae of the rough ER there are vesicles, lipid droplets and lysosomes (dense bodies). In degenerated tumor cells many lysosomes are found. Large myelin-like bodies are found to be attached to ordinary dense lysosomes. They contain phospholipid which comes from the break down of components of membrane structures in the autophagic vacuoles. Lysosomal enzymes act on them. The round or stellateshaped lipid droplets contain phospholipid, glycolipid and cholesterol. They are products of degradation of membrane systems of the mammotrophs and specially of the greatly increased ER membranes.

### PHARYNGEAL HYPOPHYSIS

Gonzalez *et al.* (1977) studied the ultrastructure of the human pharyngeal hypophysis. Four types of secretory cells could be distinguished. Type I cells have granules of about 70-90nm in diameter; Type II cells are most numerous and they are loaded with granules between 100 and 200nm. Type III cells have granules ranging between 150 and 250nm in diameter. Type IV cells have largest secretory granules of 250-400nm. Type V cell has no secretory granules and it is regarded as interstitial cell. All the cells are arranged in clusters which are surrounded by blood capillaries. Nerve fibres make synaptoid contacts with type II cells. The authors suggest that the human pharyngeal hypophysis is an endocrine gland with distinct characteristics and having a functional role probably different from that of the sellar hypophysis.



Type IV cell of the pharyngeal hypophysis resembles somatotrophs of the sellar hypophysis. They further said, "In addition, the cytology of the pharyngeal hypophysis differs from the structure that could be expected in a grafted hypophysis since lactotrophs and somatotrophs are very rare or absent in the pharyngeal hypophysis". Synaptoid contacts between nerve fibres and type II cells suggest that neural afferents control some of the functions of the pharyngeal hypophysis and thus it resembles the pars intermedia of most species or the pars distalis of teleost fishes.

# PHARYNGEAL HYPOPHYSIS

Goldman et al. (1971) studied the ultrastructure of the pharyngeal hypophysis. They found that the pharyngeal hypophysis is a distinct endocrine organ. It is located in the pharynx and is composed of several types of cells. Type I cells are the most numerous and are responsible for the production of growth hormone. Type II cells are responsible for the production of prolactin. Type III cells are responsible for the production of thyrotrophic hormone. Type IV cells are responsible for the production of somatotrophic hormone. The pharyngeal hypophysis is a distinct endocrine organ and is located in the pharynx. It is composed of several types of cells. Type I cells are the most numerous and are responsible for the production of growth hormone. Type II cells are responsible for the production of prolactin. Type III cells are responsible for the production of thyrotrophic hormone. Type IV cells are responsible for the production of somatotrophic hormone. The pharyngeal hypophysis is a distinct endocrine organ and is located in the pharynx. It is composed of several types of cells. Type I cells are the most numerous and are responsible for the production of growth hormone. Type II cells are responsible for the production of prolactin. Type III cells are responsible for the production of thyrotrophic hormone. Type IV cells are responsible for the production of somatotrophic hormone.





Tsuneki and Gorbman(1975) divided the anterior neurohypophysis of *Lampetra tridentata* into two parts, viz., rostral and caudal. Structurally, the anterior neurohypophysis has an inner ependymal layer and an outer fibre layer. The ventral part of the fiber layer, i.e. the part close to the pars distalis, has a palisade arrangement. The ependymal layer in the rostral part of the anterior neurohypophysis consists of a single layer of ependymal cells. A close study of these cells suggests that they possess absorptive and/or secretory capacities. The fiber layer in the same part consists of ependymal processes, nerve axons and axon terminals, and sometimes, glia cells. The nerve axons are of two types. While there are neurosecretory axons, there are nonsecretory axons as well, characterised by the presence of neurotubular bundles arranged loosely. Depending upon the diameter of the electron dense granules, the neurosecretory axons may be divided into three categories: (1) granules with diameter of 1400-2000 Å + small empty synaptic vesicles, (2) granules with diameter of 950-1400 Å + small vesicles and (3) granules with diameter of 650-1000 Å + small vesicles. The axons with larger granules often possess synaptoid contacts with the ependymal processes. The basal lamina has contact with the ependymal end feet as well as with the neurosecretory nerve endings. Hence the ependymal cuff is incomplete.

The general ultrastructural pattern of the caudal part of the anterior neurohypophysis is essentially similar to that of the rostral part, but there are some differences. The ependymal cells of the thin ependymal lining of the caudal part discharge large droplets into the third ventricle. The small nonneurosecretory nerve fibres are plenty in number. Axonal types are similar to those noted in the rostral part. Neurosecretory axons are less in number. Synaptoid contacts between ependymal processes and neurosecretory axons are rare, but presence of neurosecretory granules in the ependymal processes could be frequently seen. The basal lamina has contact with the ependymal end feet (light in appearance) or glial processes (dark in appearance) and thus the cuff is complete. There is no direct contact of the neurosecretory axon endings with the basal lamina and the complete cuff is interposed between them.

The ultrastructural features of the anterior neurohypophysis are similar to those of the median eminence of higher vertebrates minus the portal vessels. Tsuneki and Gorbman(1975) therefore said that the functional value of this median eminence is jeopardised, but "might represent a primitive neuroglandular relationship from which other vertebrates have evolved".

The posterior neurohypophysis may be considered equivalent to the pars nervosa of higher vertebrates and it is associated with the pars intermedia. The authors found increased accumulation of fuchsinophilic material in the pars nervosa. The blood supply is well developed for the transport of discharged neurosecretory material.



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The pharyngeal hypophysis is a small, oval, translucent structure located in the anterior part of the pharynx. It is composed of two main parts, the anterior and posterior lobes. The anterior lobe is larger and contains the majority of the secretory cells, while the posterior lobe is smaller and contains the nerve fibers and synaptoid contacts.

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## CYTOLOGY OF HYPOPHYSIS

The pharyngeal hypophysis is a small, oval, translucent structure located in the anterior part of the pharynx. It is composed of two main parts, the anterior and posterior lobes. The anterior lobe is larger and contains the majority of the secretory cells, while the posterior lobe is smaller and contains the nerve fibers and synaptoid contacts. The secretory cells are arranged in a regular pattern and are surrounded by a thin layer of connective tissue. The nerve fibers are located in the posterior lobe and are surrounded by a thin layer of connective tissue. The synaptoid contacts are located between the nerve fibers and the secretory cells, suggesting that neural afferents control some of the functions of the pharyngeal hypophysis.



## CHAPTER 7

### THE PITUITARY GLAND OF AGNATHANS

#### *Myxinoids*

Wingstrand(1966) discussed the development of the pituitary of cyclostomata. He said that the anlage of the adenohypophysis was in association with the olfactory placode and both the rudiments sink down into a common depression which later on is called the naso-hypophysial pit or canal. Subsequently the opening of this pit migrates anterodorsally around the rostrum of the embryo to the dorsal part of the head and in the adult it opened at that site. In *Myxine* the nasohypophysial opening is near the anterior end of the head and outside the mouth. "In *petromyzon* the analge of the hypophysis grows out as a compact cellular string from the naso-hypophysial pit and proliferates to form an adenohypophysis in contact with the neurohypophysis. At metamorphosis a lumen arises in the cell cord which connects the pituitary with the nasohypophysial pit. This lumen expands and extends under and behind the pituitary as a sac, which serves to ventilate the olfactory organ". This duct and sac is the lumen of Rathke's pouch but it can also be the extension of the lumen of the nasohypophysial pit. Wingstrand(1966) further said, "The fact that the lumen in the cell cord arises after the cord has lost the contact with the pituitary in *Icthyomyzon* speaks in favour of the latter interpretation. In the *Myxinoidea*, this nasopharyngeal sac opens into the pharynx. So an open nasopharyngeal duct is present".

#### *Blood supply of the median eminence, neurohypophysis and adenohypophysis of Myxinoids*

The neurohypophysis and adenohypophysis get separate blood supply from the carotid artery (Ball and Baker, 1969; Jasinski, 1969). The neurohypophysis receives an additional blood supply through the portal vessels which proceed from the prehypophysial plexus. There is separate drainage of the two parts of the pituitary by hypophysial veins.

#### *Neurohypophysis of Myxinoids*

The neurosecretory fibres from the preoptic nucleus proceed in two directions. One group ends on the capillaries of the *prehypophysial plexus* posterior to the optic chiasma.



Gorbman *et al.* (1963) and Nishioka and Bern (1966) thought that this neurohaemal contact area is similar to that of the tetrapod median eminence ultra-structurally. The neurosecretory material of the preoptic nucleus is aldehyde fuchsin negative and CAH-negative but can be stained by Astra blue. The neurons are unipolar and they have no other process to project into the third ventricle.

The second direction of the preopticoneurohypophysial fibres is towards the neurohypophysis. Few of them end around the blood capillaries at the anterior part of the neurohypophysis. Majority of the fibres end in the dorsal wall of the neurohypophysis. AF-positive neurosecretory material accumulates in this area. The axonal endings contain secretory granules of different sizes.

Sterzi (1907) and Stendell (1913) did not find apposition between the neurohypophysis and adenohypophysis in *Myxine*. Limited contact at the posterior end of the neurohypophysis in *Myxine* and *Polistotrema* was however noted by Adam (1959) and Matty (1960). A vascular plexus in front of the infundibular stalk was found by Olsson (1959) where neurosecretory axons end around the capillaries. He thought that this area is the median eminence of the hagfish. Kobayashi (1972) said that the area thought to be the median eminence by Nishioka and Bern (1966) "does not seem to be the median eminence, because the perivascular space is not thick and it has only single basement membrane unlike those of the neurohaemal organ and other endocrine organs". In these organs the perivascular space is usually thick and there are double basement membranes.

Kobayashi and Uemura (1972) considered the ventral wall of the neurohypophysis to be the median eminence of the hagfish. In the hagfish, *Eptatretus burgeri*, the fine structure was similar to that of the tetrapod median eminence. Rarely AF-positive material is seen in this location (ventral wall). The axons of the ventral wall contain three types of granules of different diameters (1100, 800 and 650 Å) and synaptic vesicle-like structures (400 Å). These axons end at the connective tissue space in between this ventral wall and the adenohypophysis. The ependymal processes are always interposed in between the axon endings and the connective tissue space.

**Ependymal cells:** In the ventral wall many bulbous protrusions occur on the apical surface of the ependymal cells. Sometimes free cytoplasmic protrusions can be found in the third ventricle. These are ependymal secretions (Kobayashi, 1972). Colloid-like droplets are present in the ependymal cells and processes. They are released into the connective tissue space. Large irregular type of vesicles in the apical portion of the cell body are only found in the ventral wall and not in the ependymal cells of the dorsal wall. Kobayashi (1972) thinks that this vesicle formation has something to do with the adenohypophysial function as they are only found in the ependymal cells of the ventral wall. Many vesicles with diameter of 600 to 1000 Å in the ependymal cell body and processes can be noted and these are formed in the Golgi apparatus and proceed along the process.



They accumulate and attach to the terminal membrane of the processes just like pinocytosis. As the vesicles seem to be formed in the Golgi apparatus, they may be exocytosis. This type of vesicle formation was not noted in the ependymal cells of the median eminence of higher vertebrates. Its physiological importance is unknown at present. Monoaminergic axons have been noted in the neurohypophysis. There are synaptoid contacts between some of these axons with the ependymal perikarya or processes. Kobayashi(1972) is of the opinion that in the hagfish and higher vertebrates the ependymal function may be regulated by these monoaminergic fibres.

*Dorsal wall* : Plenty of AF-positive material is seen. Large granules (2,900 to 4,000 Å) as well as smaller ones (1,100, 800, and 650 Å) exist in the axons. The large-granule-group may carry neurohypophysial hormone. The ependymal cells do not contain large irregular-shaped vesicles. Colloid droplets are smaller in number in comparison to those in the ventral wall. The ependymal cells contain vesicles of 600 to 1,100Å. Capillary plexus is well developed on the surface of the dorsal wall. On the surface of the ventral wall the capillaries are very small in number. About 20 vessels connect the ventral wall with the adeno-hypophysis. Blood flows from the median eminence (ventral wall) to the adeno-hypophysis and so these are portal vessels and the venous drainage from the adeno-hypophysis is to the hypophysial vein (Kobayashi and Uemura, 1972).

In *Polistotrema* and *Myxine* few or no portal vessels course through the connective tissue (Jasinski, 1969; Fernholm, 1972).

#### *Adenohypophysis of Myxinoids (figs. 7.1 and 7.2)*

The adenohypophysial cells are arranged in clusters and follicles. They are situated in the thick connective tissue between the neurohypophysis and the nasopharyngeal duct. The adenohypophysis is not regionally subdivided. The colloid in the follicles is PAS-positive, aldehyde fuchsin-positive, alcian blue-positive and aniline blue-positive but most of the cells are situated in compact clusters. The blood supply is poor. No nerve fibre can be found in the adenohypophysis.

Fernholm and Olsson(1969) could identify PAS-positive and aldehyde fuchsin-positive basophils of different types, several types of chromophobes, and erythrosinophils of two types : one with coarse and the other with fine granules. They could attach corticotrophic function to the finely granulated erythrosinophils as these cells responded to adrenocortical blocking agents. LTH secretion by the coarsely granulated erythrosinophils could not be confirmed by Sage and Bern (1972)(bioassay) or by Aler *et al.*(1971) (immunochemical methods).

Sandor *et al.*, (1976) discussed the biosynthesis of corticosteroids throughout the vertebrates and Sandor(1979) reviewed the comparative aspects of steroid biosynthesis in fish. Idler and Burton(1976) thought that the pronephroi are the sites of presumptive adrenal cells in the hagfish, *Myxine glutinosa* L. Chester





Jones and Phillips(1960) and Phillips *et al.*, (1962) found cortisol and corticosterone in the plasma of the Atlantic hagfish, *Myxine glutinosa*. Idler *et al.*(1971) reported the presence of cortisol, cortisone and corticosterone in the Atlantic hagfish and the steroids increased after mammalian ACTH treatment, when 11-deoxycorticosterone was also found.

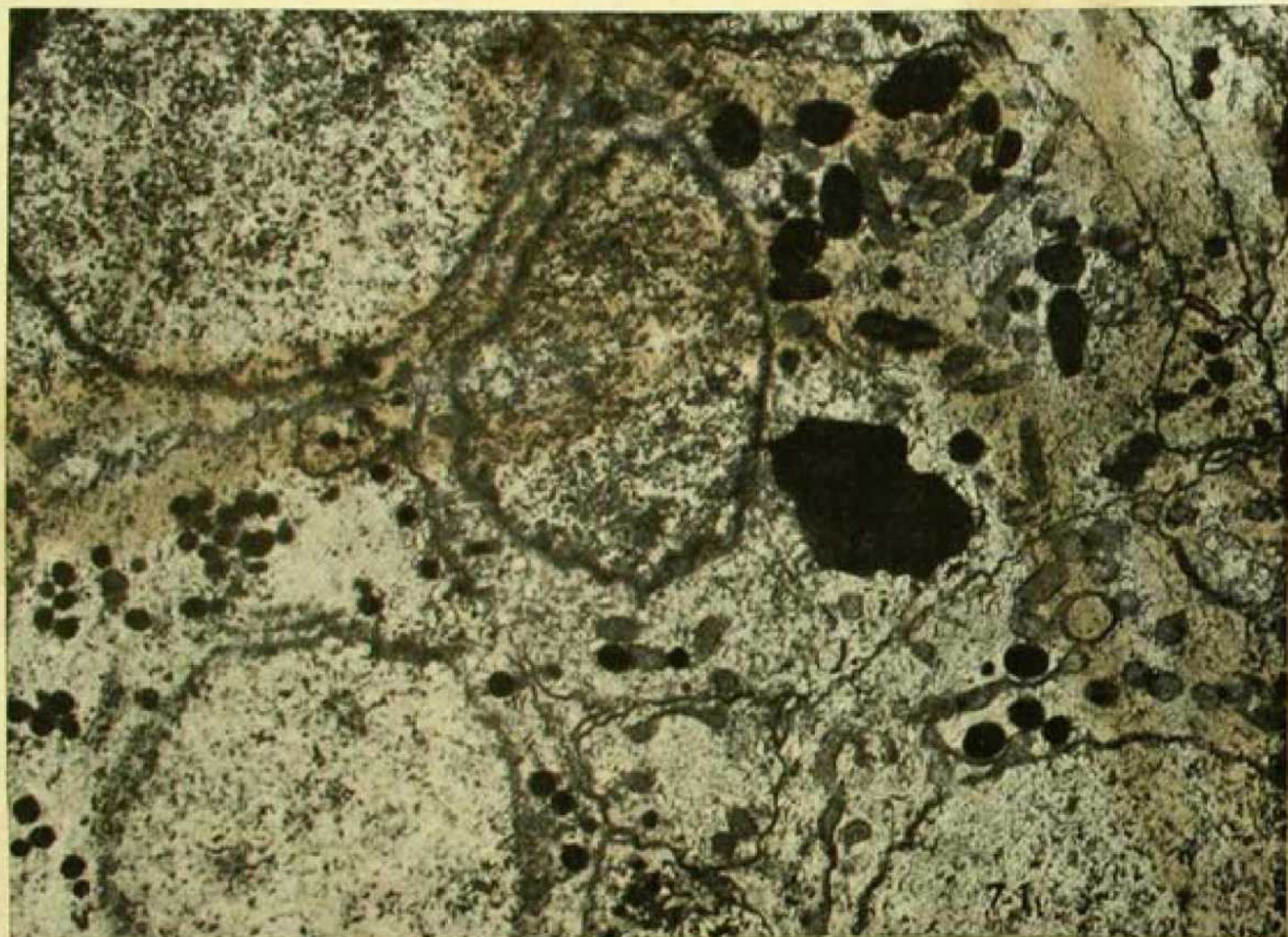


Fig. 7.1.

Fernholm(1972) identified two cell types secreting protein hormones in the *Myxine* adenohypophysis with the help of electron microscope. Type I cells had secretory granules of 88nm in diameter and a hormone is being secreted similar to ACTH/MSH. Type II cells had granules of 176nm in diameter and probably secrete STH/LTH. This prolactin has not the same antigenic properties as mammalian prolactin (Aler *et al.*, 1971). Fernholm thought that each cell type does not secrete two different hormones. Instead "each cell type produces a molecule resembling the parent molecule of one of the two protidic hormone families (ACTH-MSH-lipotropin and STH-LTH)". Ultrastructurally the PAS-positive basophil cells could not be separated into different types. The ultrastructure is different from known thyrotrophs and gonadotrophs of other vertebrates. These cells are akin to mucous cells of the skin of the hagfish which



have been included into the pituitary but transformation into endocrine cells have not occurred. No proper intermediate lobe can be detected.

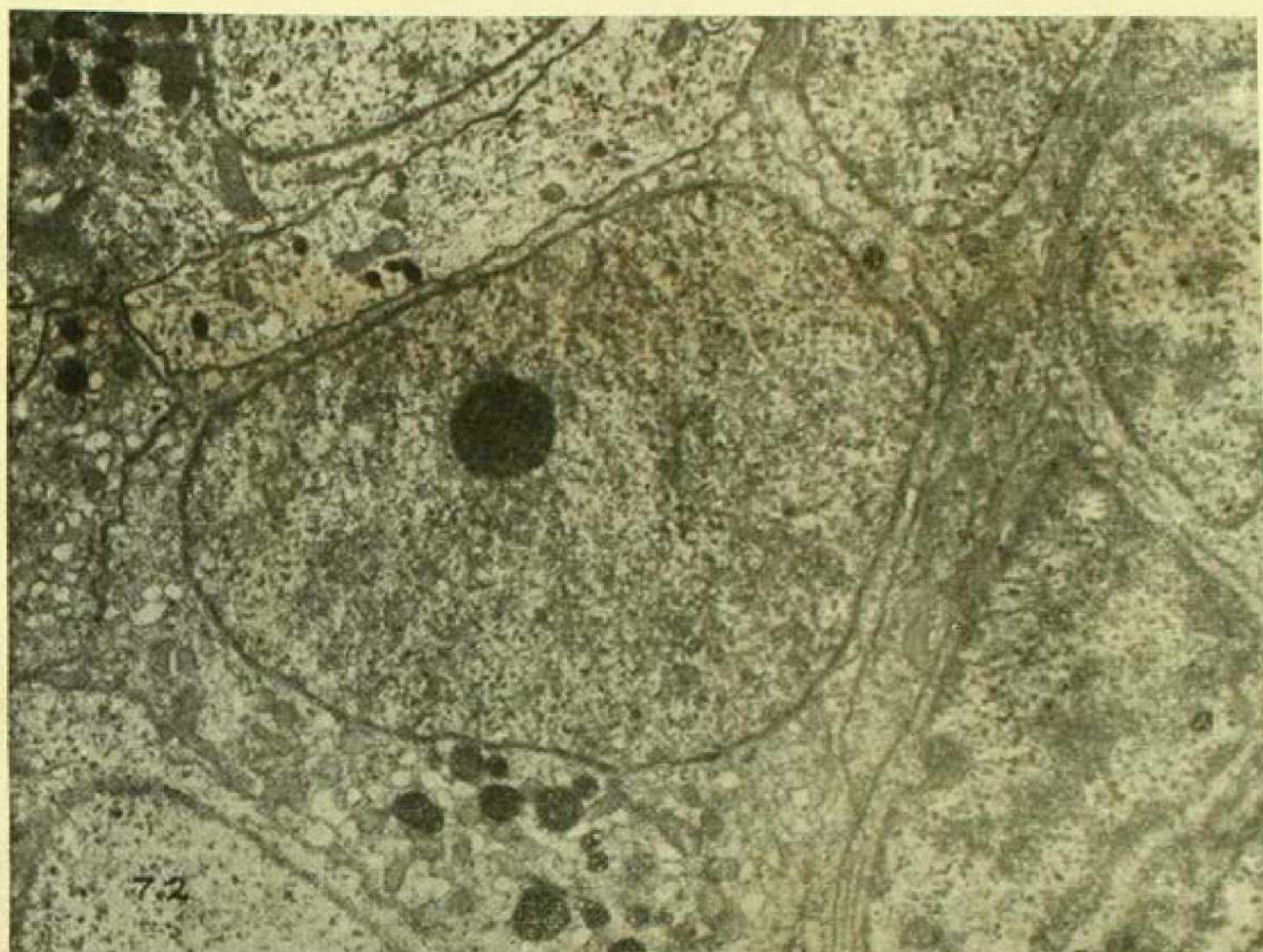


Fig. 7.2.

Figs. 7.1 & 7.2 Adenohypophysis of the hagfish, *Eptatretus burgeri*. In this primitive cyclostome, secretory granules are not abundant. Only a few types of granulated cells are found. Large irregular granules in the picture are possibly lysosomes. Only small round granules are secretory. Functional identification of cell types is impossible. Figs. 7.1 & 7.2 : Courtesy of Dr. Kobayashi and Dr. Tsuneki(1977).

Tsuneki(1976) studied the effects of estradiol and testosterone in the hagfish *Eptatretus burgeri*. In the ovary estradiol caused degeneration (pharmacological effect but testosterone had no effect on the ovary. These steroids had no significant effect on the testis. Conspicuous change in the adenohypophysial cells was not noted after administration of the steroids. Therefore, a feedback mechanism between gonadal steroids and gonadotrophin either does not exist or plays a very minor role. In the Atlantic hagfish, *Myxine glutinosa* some adenohypophysial cells show reactions to high doses of sex steroids (Fernholm and Olsson, 1969). Fernholm(1972) could not find glycoprotein hormone-secreting cells ultrastructurally in the adenohypophysis of *Myxine*. 3 $\beta$ -hydroxy-



steroid dehydrogenase histochemical activity was not seen in the ovary of *Myxine* (Fernholm, 1972). Gorbman and Tsuneki(1975) observed gonadal atrophy after hypophysectomy in the Pacific hagfish, *Eptatretus*. Hirose *et al.*(1975) found production of steroid hormones by the mature ovary of *Eptatretus burgeri*.

Tsuneki(1976) observed that with Herlant's tetrachrome and PAS-orange G-alcian blue stainings the adeno-hypophysial cells of *Eptatretus burgeri* in the experimental series did not show any specific stainability. The cytoplasm of some adeno-hypophysial cells was stained slightly by paraldehyde fuchsin. After hormone administration Tsuneki(1976) did not find any change in the hypothalamic neurosecretory neurons.

Ultrastructurally adeno-hypophysial cells were poorly granulated. Many electron-dense round structures are probably lysosomes. Three types of granulated adeno-hypophysial cells could be observed. Type I cells contain large granules (220 to 310 nm in diameter). The granules of type II cells are 170 to 220 nm in diameter. Type III cells (few in number) have small granules(100 to 170 nm). Stellate cells, highly vacuolated cells, cells with many lysosomes of different sizes and shapes, and cells containing a small amount of cytoplasm with few organelles were also met with. In the interfollicular connective tissue blood vessels were occasionally found. In some of the testosterone treated animals mature testicular follicles could be correlated with intense accumulation of types I and II cells, specially type I cells. Cytoplasmic activity in these cells was not reduced and the cytoplasm was occasionally well-vacuolated. Tsuneki(1976) concludes, "It is probable that the feedback mechanism between the pituitary and the gonad does not effectively operate in the course of maturation in the cyclostomes. The present results obtained from *Eptatretus* may support Fernholm's conclusion(1972) that the pituitary-gonadal axis is not functional in the hagfish, *Myxine*".

Holmes and Ball(1974) said, "the myxinoid pituitary is genuinely primitive, representing an evolutionary stage before the development of thyrotrophs and gonadotrophs, and before the differentiation of ACTH from MSH and of LTH from STH".

The hormones are carried slowly through the connective tissue to blood vessels (Fernholm and Olsson, 1969) and ependymal cells with long processes have been detected in the cell clusters. These cells have microtubules and may function as *stellate cells* for mobilization of the hormones.

#### *Petromyzontids*

##### *Blood supply of the neural lobe and pars distalis*

Only a vascular septum is interposed between the neural lobe and the metaadeno-hypophysis. It receives branches from the internal carotid to the plexus intermedius in this location. It drains into an independent hypophysial vein (Gorbman, 1965). Several small branches of the internal carotid artery supply the pars distalis. It is drained by small veins.



*Neurohypophysis*

It is not definitely known whether the lampreys have a median eminence and portal vessels. Holmes and Ball(1974) state that a few preopticoneurohypophysial fibres end in the vascular connective tissue sheet between the hypothalamus and the rostral pars distalis. This area corresponds to the median eminence of higher vertebrates. Jasinski(1969) could trace a few blood vessels from this place to the rostral pars distalis. In the pars distalis "the capillaries ascend between cell cords and descend as wider sinusoids". The loop is situated dorsally close to the *contact area* where there are some fibre endings of the preoptic nucleus (Gorbman, 1965).

No connective tissue cells have been noted in the neural lobe of the lamprey by Rodriguez(1971).

Wingstrand (1966) said that there is no anatomical separation between the median eminence and neural lobe in the neurohypophysis of Lamprey. Different types of fibres could be distinguished by Rodriguez(1971) by electron microscope. Some of these fibres were like those seen in the median eminence of other species while others would represent neural lobe fibres. Fibres containing granules larger than 130nm were neural lobe fibres. He could find a zonation in the Lamprey neurohypophysis as there are areas where there is predominance of the neural lobe fibres. The majority of neural lobe fibres are of a single type called typeIII. The granules are of irregular shape and of high electron density and ranges in size from 150 to 180nm. Many are elongated and the length is 300-500nm. Granules of lesser density than the previous ones, more spherical with a diameter of 150 to 180 nm are contained in typeIV axons. This type is few in number. TypeII endings are plenty in number containing granules smaller than 100nm. TypeI endings contain only small vesicles.

Rodriguez(1971) postulated that the 150-180 nm granules store arginine vasotocin(AVT). In the lamprey neurohypophysis a few fibres containing granules other than 150 to 180nm granules present in the *vasotocinergic* fibres indicate that more than one neurohypophysial hormone are being elaborated. Sawyer *et al.*(1961) and Follet and Heller(1964) found the lamprey neurohypophysis to contain AVT only. They suggested the possibility of the presence of a second principle.

The neural lobe of the lamprey has a dorsal ependymal layer, a fibre layer and a ventral palisade layer which contains AF+nsf and approximated to the neurointermedia septum. The nsf of the preoptic nucleus is AF-positive and CAH-positive. The cells have projection of short dendrites which proceed towards the CS fluid after coursing between the ependymal cells.

Gorbman(1965) did not find blood vessels from the hypothalamus to the pars distalis in the lamprey. Sterba(1969) found that environmental stimuli could lead to different varieties of seasonal physiological phenomena in the lamprey through the central nervous system acting on the adenohypophysis.





Tsuneki and Gorbman(1975) divided the anterior neurohypophysis of *Lampetra tridentata* into two parts, viz., rostral and caudal. Structurally, the anterior neurohypophysis has an inner ependymal layer and an outer fibre layer. The ventral part of the fiber layer, i.e. the part close to the pars distalis, has a palisade arrangement. The ependymal layer in the rostral part of the anterior neurohypophysis consists of a single layer of ependymal cells. A close study of these cells suggests that they possess absorptive and/or secretory capacities. The fiber layer in the same part consists of ependymal processes, nerve axons and axon terminals, and sometimes, glia cells. The nerve axons are of two types. While there are neurosecretory axons, there are nonsecretory axons as well, characterised by the presence of neurotubular bundles arranged loosely. Depending upon the diameter of the electron dense granules, the neurosecretory axons may be divided into three categories: (1) granules with diameter of 1400-2000 Å + small empty synaptic vesicles, (2) granules with diameter of 950-1400 Å + small vesicles and (3) granules with diameter of 650-1000 Å + small vesicles. The axons with larger granules often possess synaptoid contacts with the ependymal processes. The basal lamina has contact with the ependymal end feet as well as with the neurosecretory nerve endings. Hence the ependymal cuff is incomplete.

The general ultrastructural pattern of the caudal part of the anterior neurohypophysis is essentially similar to that of the rostral part, but there are some differences. The ependymal cells of the thin ependymal lining of the caudal part discharge large droplets into the third ventricle. The small nonneurosecretory nerve fibres are plenty in number. Axonal types are similar to those noted in the rostral part. Neurosecretory axons are less in number. Synaptoid contacts between ependymal processes and neurosecretory axons are rare, but presence of neurosecretory granules in the ependymal processes could be frequently seen. The basal lamina has contact with the ependymal end feet (light in appearance) or glial processes (dark in appearance) and thus the cuff is complete. There is no direct contact of the neurosecretory axon endings with the basal lamina and the complete cuff is interposed between them.

The ultrastructural features of the anterior neurohypophysis are similar to those of the median eminence of higher vertebrates minus the portal vessels. Tsuneki and Gorbman(1975) therefore said that the functional value of this median eminence is jeopardised, but "might represent a primitive neuroglandular relationship from which other vertebrates have evolved".

The posterior neurohypophysis may be considered equivalent to the pars nervosa of higher vertebrates and it is associated with the pars intermedia. The authors found increased accumulation of fuchsinophilic material in the pars nervosa. The blood supply is well developed for the transport of discharged neurosecretory material.



*Lamprey pars distalis (fig 7.3)*

In ammocoetes and adult lampreys the adenohypophysis can be divided into two parts: the pars distalis (anteriorly) and the pars intermedia (posteriorly). The pars intermedia (metaadenohypophysis) is situated below the neurohypophysis, and the two are separated by basal membranes and a thin capillary plexus. The pars distalis is situated under the post-optic hypothalamic floor, and is separated from this structure as well as the pars intermedia by a thick layer of connective tissue. The pars distalis is divided into several lobules by dorsoventrally oriented connective tissue septa and capillaries, and it is also divided into two zones: rostral (proadenohypophysis) and caudal (mesoadenohypophysis).

The account of the pituitary gland of the brook lamprey (*Lampetra planeri*) before, during and after metamorphosis as described by van de Kamer and Schreurs (1959) is the basis of modern histological studies. It has also been stressed by van Oordt (1968). The *proadenohypophysis* consists of aniline blue + basophils in small larva. They are elongated cells with one pole directed towards the capillary situated under the infundibular wall. The cytoplasm contains a number of small droplets of secretion. In older larvae the number of basophils are increased and before metamorphosis majority of the cells are basophilic. Most intense basophilia has been observed in the cytoplasm of cells situated more dorsally than in the ventrally located cells and thus a staining gradient (dorsoventral) is obtained. Tsuneki and Gorbman (1975) relate this gradient to the distribution of neurogenic factor. It reflects "a regional difference in diffusion rate and concentration of hypothalamic factors". Surface enlargement is produced by the connective tissue septa which divide this part of the gland into different portions. The elongated parts of the basophils terminate on the connective tissue septa. The secretory droplets are extruded into the surrounding connective tissue. During metamorphosis the *proadenohypophysial* cells are strongly basophilic (AB+) and they are also PAS+. After metamorphosis the secretion diminishes and after spawning the cells shrink with few signs of activity. The *proadenohypophysial* basophils were thought to be the source of gonadotrophic hormone as the cytological changes were associated with growth of ovaries, vitellogenesis and spawning. Differentiation of *mesoadenohypophysial* cells occurred much later. At metamorphosis two cell types could be observed. The first and dominant type is a large chromophobic cell. It may be elongated or oval. The second type is an irregular, triangular or polyhedral basophilic cell, scattered among the chromophobes. The granules in these cells are AB+ and PAS+. These cells may produce thyrotrophic hormone. The somatotrophs are also situated in the *mesoadenohypophysis*. The *metaadenohypophysis* contains elongated cells having a basal part where the nucleus is situated. The apical part is slender and borders the blood vessels between the neurohypophysis and the metaadenohypophysis. Abundant granular secretion has been noted in the apical part and this stains with azocarmine and is PAS+. These cells produced melanotrophin. The water-balance factor is presumably present in the neurohypophysis.



Roth(1957) studied the pars distalis of the adenohypophysis of the sea lamprey, *Petromyzon marinus*. In the young ammocoete no secretory granules could be detected in the cells. In subsequent stages of development five cell types could be found. There were two types of chromophobes and one acidophilic type. The remaining three cell types described by Roth were not the same as observed by van de Kamer and Schreurs(1959).

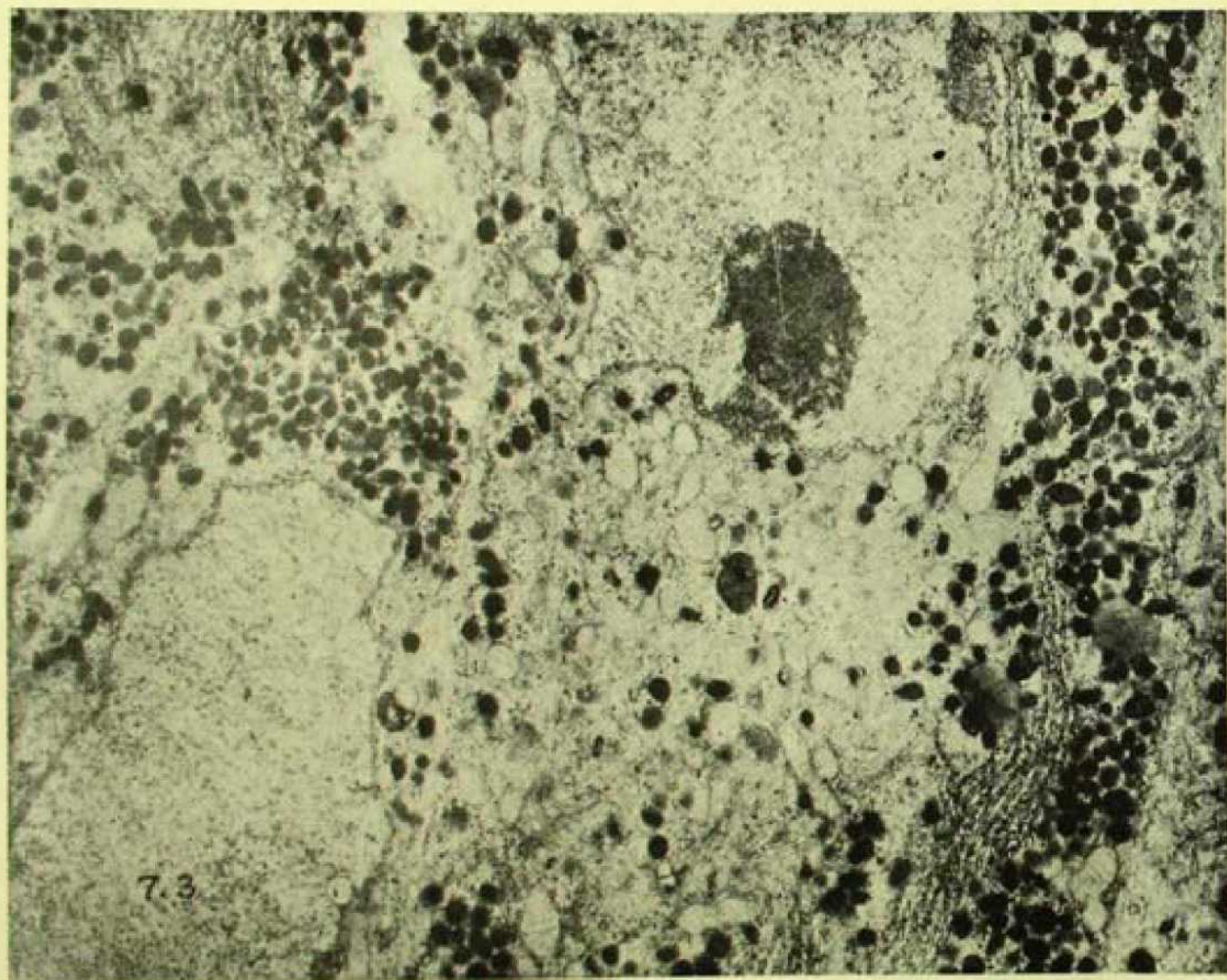


Fig. 7.3. Highly granulated cells in the rostral pars distalis of the lamprey, *Lampetra japonica*. In this cyclostome, several cell types with abundant secretory granules are found in the adenohypophysis. The cell type shown in the picture is basophilic at LM level. This may be a gonadotroph. Courtesy of Dr. Kobayashi and Dr. Tsuneki(1977).

van Oordt(1968) said that the granules in the basophilic cells of the pars distalis and the carminophils of the pars intermedia are generally found to be stainable with Gabe's aldehyde fuchsin. The basophils of the proadenohypophysis are AF+ and PAS+. They may contain PAS-negative material which can be stained by erythrosine, orange G, iron haematoxylin(Ruhle and Sterba, 1966) and lead haematoxylin(Larsen and van Oordt, 1966). Groups of smaller chromophilic cells with occasional basophilic granules may also be found in the proadenohypophysis in addition to the basophils.



The mesoadenohypophysis of river lampreys caught in spring contains strongly carminophilic and iron haematoxylin+ cells with scanty cytoplasm (Ruhle and Sterba, 1966). These cells are situated along the blood vessels and connective tissue septa. Larsen and van Oordt identified similar small cells and they were found to be scattered throughout the mesoadenohypophysis of *Lampetra fluviatilis*. These cells had a tendency to concentrate at the periphery of tissue lobules at the dorsal part of this zone. They stain violet in Herlant's tetrachrome and are strongly lead haematoxylin +. Mesoadenohypophysial chromophobes are often elongated and have a follicular arrangement. The metaadenohypophysial cells are weakly PAS+ or PAS-. These cells contain lead haematoxylin + granules (Larsen and van Oordt, 1966).

Cysts and pseudofollicles have been observed in the adenohypophysis of lampreys and these are particularly common in parasitized specimens. van Oordt (1968) said, "since these structures closely resemble cysts arising from a disruption of the portal circulation in higher vertebrates, they might also in lampreys be a sign of insufficient drainage of the adenohypophysis."

Lanzing (1959) and Ruhle and Sterba (1966) suggested that basophils of the proadenohypophysis produced gonadotrophin and mesoadenohypophysial basophils produced TSH. Evennett (1963) and Evennett and Dodd (1963) thought that the mesoadenohypophysial basophils secrete gonadotrophin. From the results of partial hypophysectomy Larsen (1965) concluded that gonadotrophin is produced in the mesoadenohypophysis and probably also in the proadenohypophysis. After extirpation of the pro- and mesoadenohypophysis secondary sexual characters develop sporadically. Release of sperm into the body cavity is not found in males, but spermatogenesis goes on with production of spermatozoa. Ovulation does not occur in females, but growth of eggs continues, although at a reduced level compared with normal females. Larsen (1965) further stated, "In males appearance of secondary sexual characters and release of sperm into the body cavity seem to depend on the same gonadotrophic hormone, but in females it is suggested that two different hormones are secreted, one inducing egg growth and secondary sexual characters, and the other being necessary for ovulation."

Larsen (1973) discussed possible factors involved in initiation of sexual maturation in river lampreys, *Lampetra fluviatilis*. She concluded that initiation of sexual maturation cannot be achieved by a rise in the temperature of the water or by an increase in the length of the day (Larsen and Rothwell, 1972). A hypothalamic control might act according to some process not related to the environmental conditions. This was proved by pituitary autotransplantation ( $1/8-1/2$  of the gland to a pharyngeal muscle) experiments. Treatment with gonadotrophin cannot produce a clearcut precocious sexual maturation in intact lampreys (Larsen and Rothwell, 1972).

Endostyle of the lamprey larva is engaged in typical thyroidal biosynthesis before its transformation into the thyroid at metamorphosis (Barrington, 1968).



He further said, "the follicular structure of the thyroid gland may thus be an adaptation to freshwater life, providing as it does for ample storage of the iodinated hormones, and for their release as the need arises. Incidentally, it would be at this stage, rather than at the stage of intracellular storage that the establishment of pituitary control of thyroidal function would have been adaptively advantageous."

From the observation of Knowles(1941), Barrington and Sage(1966), Larsen and Rosenkilde(1971) and Pickering(1972) it has been found that the functions of the endostyle and thyroid gland seem to be independent of the pituitary.

Honma(1969) found that acidophils and basophils are arranged in vertical cell cords in the proximal pars distalis. In the Transylvanian lamprey *Eudontomyzon* there are acidophils in the proximal pars distalis which are carminophilic after Azan, weakly Alizarin Blue-positive, and strongly lead haematoxylin-positive. With aldactone treatment there was degranulation and vacuolation of these cells. Molnar and Szabo(1968) suggested that these cells are corticotrophs. LTH is not secreted by acidophils (Aler *et al.*, 1971). The PAS-positive and aldehyde fuchsin-positive basophils of this location and of the rostral pars distalis seem to be gonadotrophs.

Pars intermedia cells are large and secrete MSH(Larsen and Rothwell, 1972 van Oordt, 1968; Ball and Baker, 1969). Majority of the intermedia cells are chromophobic but some are chromophilic. The latter type is carminophilic. Depending upon the type of species they may be PAS-positive or PAS-negative and lead haematoxylin-positive as in *Lampetra fluviatilis* (van Oordt, 1968). Occasional basophil cells were found in the pars intermedia of the river lamprey by Ruhle and Sterba(1966). Vacuolization of the cytoplasm of adenohypophysial cells is found when there is extrusion of secretory products.

Hypophysial extract from the lamprey showed marked corticotrophic activity when tested on mice(Strahan, 1959) and on toads(Larsen and Rothwell, 1972). Strahan(1962) found evidences for the existence of ACTH in the hag and lamprey pituitaries. Thirty whole hag pituitaries could significantly increase the weight of the adrenals of immature female mice. The pituitary gland of the lamprey is much more potent, 5 glands being sufficient to give a significant response. Increase in uterine weight in immature mice could be achieved by both lamprey and hag pituitaries, but there was no noticeable ovarian response. Thyrotrophic effect could not be demonstrated also. Jorgensen(1976) reviewed the submammalian vertebrate hypothalamic-pituitary-adrenal interrelationships. Molnar and Szabo(1968) treated the Transylvanian lamprey(*Eudontomyzon danfordi*) with aldactone. There was degranulation of the lead haematoxylin positive(ACTH) cells. Larsen and Rothwell(1972) noted similar effects in *L. fluviatilis* after metyrapone treatment. Negative feedback interrelations exist between interrenal tissue and hypophysis. Interrenal tissue in *L. fluviatilis* is functionally comparable to the adrenocortical tissue of other vertebrates(Hardisty, 1972). Jorgensen(1976) said, "In summary, it seems that if hypophysial-inter-



renal functional interrelations do exist in cyclostomes they are less developed and differentiated than in higher vertebrates."

The ultrastructure has been described briefly by Bage(1971) and Larsen and Rothwell(1972). Tsuneki and Gorbman(1975) described the structure of the pars distalis of the lamprey, *Lampetra tridentata* in details. The pars distalis can be divided into rostral and caudal parts. Three types of secretory cells have been noted in the rostral pars distalis: (1) intensely chromophilic cells stained greenish violet; (2) large round cells stained weakly with AF; and (3) chromophobic cells (less in number). The cells of the first type are more frequently found. The two lateral lobes of the caudal pars distalis proceed anteriorly and partly surround the rostral pars distalis from the sides. The cells are arranged in cords as is found in the rostral pars distalis. The caudal pars distalis contains three cell types: (1) chromophobic cells are abundant; (2) intensely green cells are infrequent; and (3) round cells intensely stained with AF.

Ultrastructurally there are three types of secretory cells in addition to non-secretory cells in the rostral pars distalis. The nonsecretory cells are less frequent. The slender cytoplasmic processes extend among the secretory cells. These *stellate cells* are also found in the pars intermedia of the lamprey and more frequently in the caudal pars distalis. In the rostral pars distalis type I cells (granule diameter 2300-5000 Å) are plenty in number. This cell type may correspond to the most highly basophilic cells. The diameter of the granules of type II cells is 1100-2000 Å. The granules of type III cells are 2800-3800 Å in diameter and this cell type possesses high synthetic activity. The type VII cells which are normal inhabitants of the caudal pars distalis may also occur here. The caudal pars distalis contains five types of granulated cells and two types of nongranulated cells. The diameter of the granules of different cell types is as follows: type IV-2400 to 3000 Å; type VI-1100 to 1800 Å; type VII-1800 to 2400 Å; type VIII-1500-2500 Å; type IX-1200-1800 Å. Type IV cells may correspond to the basophilic cells. Type V cells are agranular and show well developed vacuolar system. Type VI is the most frequent cell type in this location. The stellate cells are also agranular cells.

The authors did not find any nerve fibre in the pars distalis. Vascular connections between pars distalis and brain could not be observed.

Lofts and Bern(1972) and Weisbart and Youson(1975) thought that the presumptive adrenal tissue is situated in the pronephric part of the sea lamprey, *Petromyzon marinus*. Plasma corticosteroids could not be detected in *Petromyzon marinus* by Weisbart and Idler (1970). Weisbart and Youson(1975) found 11-deoxycortisol, 17 $\alpha$ -hydroxyprogesterone and androstenedione in incubation studies of presumptive adrenal tissue with ( $^{14}$ C) progesterone in the larval and adult parasitic sea lamprey. No steroid could be detected in sexually mature form of sea lamprey after incubation of presumptive adrenal tissue with ( $^3$ H) cholesterol (Weisbart *et al.*, 1978). Steroids could not be detected in the peripheral blood





of *Lampetra fluviatilis* (river lamprey) (adult females) with or without mammalian ACTH (Buus and Larsen, 1975).

Sandor (1979) therefore stated, "It can be accepted that Agnatha most probably produce adrenocorticosteroids of the cortisol-cortisone-corticosterone types."

Weisbart *et al.* (1980) could identify cortisol, 11-deoxycortisol, corticosterone, 11-dehydrocorticosterone, and testosterone in the sera of the Pacific hagfish, *Eptatretus stouti*, and of the sea lamprey, *Petromyzon marinus*, by double-isotope derivative assays (DIDA). 17 $\alpha$ -Hydroxy-20 $\beta$ -dihydroprogesterone was rigorously identified in the serum of ACTH-treated sea lamprey. In either fish cortisone or 11-deoxycorticosterone could not be found. By a radioimmunoassay method, circulating levels of cortisol, 11-deoxycortisol, and corticosterone were also measured in individual hagfish. Multiple injections of ACTH consistently elevated serum concentration in hagfish. Effects of ACTH on other steroids were variable.

Crim *et al.* (1979) studied the distribution of immunoreactive luteinizing hormone (ir-LHRH) in brains of agnathan fishes (comparisons of adult Pacific Lamprey, *Entosphenus tridentata* and the Pacific hagfish, *Eptatretus stouti*). LHRH+cells were noted in the preoptic nucleus of lampreys. Beaded axons having Herring body-like dilatations were stained and found to pass ventro-posteriorly into the infundibulum where presumed nerve endings were noted in the neurohypophysis. Vasotocinergic cell bodies are AF+, but the ir-LHRH+cell bodies are distinct from them. LHRH-negative areas are other parts of the lamprey brain, and any part of the hagfish central nervous tissues. The authors could not find any way of transfer of ir-LHRH from hypothalamus to the pars distalis in lamprey. "However, detection of mammalian-like ir-LHRH in brain of this primitive agnathan fish indicates that this peptide may be of great evolutionary antiquity."

With advanced reproductive status the Western brook lamprey (*Lampetra richardsoni*) showed greatest distribution of ir-LHRH (Crim *et al.*, 1979). By radioimmunoassay whole brain extracts of lamprey and hagfish have been shown to contain ir-TRH (Jackson and Reichlin, 1974), and ir-somatostatin (Vale *et al.*, 1976) respectively. Holmquist *et al.* (1979) characterized cholecystokinin-like peptides immunochemically in lamprey gut and brain. Dickhoff *et al.* (1978) obtained lack of effect of synthetic thyrotrophin releasing hormone in Pacific hagfish (*Eptatretus stouti*) pituitary-thyroid tissues *in vitro*. Treatment with TRH could not show pituitary related actions in myxinooids *in vivo* (Tsuneki and Fernholm, 1975).

TRH is present in the head end of the amphioxus (Jackson and Reichlin, 1974). Grimm-Jorgensen *et al.* (1975) noted the presence of immunoreactive thyrotrophin releasing factor in gastropod circumoesophageal ganglia. Jackson (1978) said, "As the lamprey lacks TSH and the amphioxus and snail lack a pituitary, we suggest that the TSH-regulating functions of TRH may be a late evolutionary development representing an example of an organism acquiring a new function for a preexisting chemical substance or hormone, analogous to the



evolution of neurohypophysial hormones. In a sense, the pituitary has co-opted TRH as a regulatory hormone."

Crim *et al.* (1979) immunocytochemically located LHRH in brains of agnathan fishes. Patterns of immunoreactivity in larval and maturing Western brook lamprey (*Lampetra richardsoni*) have been described by them. In *larval animals* faint specific LHRH + areas corresponded to the posterior preoptic nucleus, fibre tracts passing ventro-posteriorly into the infundibulum and the nerve endings in the neurohypophysis. In *non-reproductive adults* faintly stained LHRH + cell bodies were found throughout the preoptic nucleus. Intense staining of neurohypophysis was obtained. In *reproductive adults*, heavy staining was observed in cell bodies of preoptic nucleus, in beaded axons which contained Herring body-like dilatations and in the neurohypophysis. Aldehyde fuchsin + vasotocinergic cell bodies were distinct from ir-LHRH cell bodies. Other brain areas and control preparations showed negative response. The immunoreactivity increased with metamorphosis and sexual maturation. Mechanisms of transport of LHRH from preoptico-neurohypophysial system to the pars distalis could not be established by the authors.



## CHAPTER 8

### THE PITUITARY GLAND OF THE ELASMOBRANCHIOMORPHS

Wingstrand(1966) discussed the development in details. The elasmobranchs have a saccus vasculosus which is developed from the embryonic saccus infundibuli. The *pars intermedia* is situated below the neural lobe and intimately fused with it to form the neuro-intermedia. The neural lobe interdigitates or ramifies within the intermedia. Variation in the intermingling of the two components is noted. The elongated *pars distalis* is divided into a *dorsal* and a *ventral lobe*. The dorsal lobe has a head and a tail. The narrow rostral part is called the head and the wide caudal part is called the tail. The dorsal lobe extends forwards beneath the infundibular floor. An epithelial stalk connects the *ventral lobe* with the tail of the dorsal lobe. The epithelial stalk is hollow in the embryo (Alluchon-Gerard,1971). A hollow and complicated Rathke's pouch gives rise to the adenohypophysis including the ventral or inferior lobe. The ventral lobe is detached from the main gland. In sharks Mellinger(1960) and Meurling(1960) described a typical median eminence and a portal circulation.

The *pars distalis* is developed from a hollow Rathke's pouch (fig. 8.1) but the anlage is very much complicated. The top of the pouch attached to the saccus infundibuli gives rise to the *pars intermedia* and it includes most of the aboral lobe. A pair of lateral lobes bends downwards and fuse below the main gland and forms the ventral lobe. The lateral lobes of the tetrapods do not bend downwards. The ventral lobe is separated from the main gland. Part of *pars distalis* is formed by that part of Rathke's pouch which is situated between the epithelial stalk and the *pars intermedia*. It is mainly developed by a large anterior process (Vorraum of Woerdeman, 1914). "This process is folded off from the oral ectoderm later than the other parts, but since this is true also for the anterior process of tetrapods, the homology appears to be well founded". The tail of the dorsal lobe is formed from part of the aboral process of Rathke's pouch. Wingstrand(1966) and Alluchon-Gerard(1971) thought that the ventral lobe seemed to be homologous with the tetrapod *pars tuberalis*. In the sharks the ventral lobe is situated in the ventrocaudal corner of the sella. In the rays, it is a flattened body which is situated more anteriorly and sometimes below the chizma. In adult holocephalians the dorsal lobe cannot be discerned to have a head and a tail and in place of the ventral lobe, they have a follicular glandular structure called the *Rachendachhypophyse*. This is far anteriorly situated, outside the cranium and in the roof of the oral cavity. The ventral lobe is homologous with the *pars tuberalis* "and the different fate of the anlage in later stages can hardly weaken this conclusion, but some doubt has been expressed (Gorbman and Bern, 1962; Diepen, 1962)." The *Rachendachhypophyse* in young *Chimaera*



*monstrosa* is connected to the head of the dorsal lobe by an epithelial cord (Honma, 1969). In *Hydrolagus coliei* the Rachendachhypophyse is developed as an evagination from the oral part of Rathke's pouch and not from the lateral processes which form the ventral lobe and pars tuberalis. Honma(1969) stated that in young *Chimaera* an epithelial stalk of Rathke's pouch connects the anlage of Rachendachhypophyse to the head of the dorsal lobe. There is gradual disappearance of this stalk with the growth of cartilage which separates the Rachendachhypophyse from the pars distalis. Holmes and Ball(1974) stated, "Thus, the Rachendachhypophyse is not embryologically comparable with the saccular ventral lobe, and its development suggests instead a tentative homology with the buccal part of the pituitary of the coelacanth, *Latimeria*; both structures may simply represent a detached piece of the rostral tip of the pars distalis."

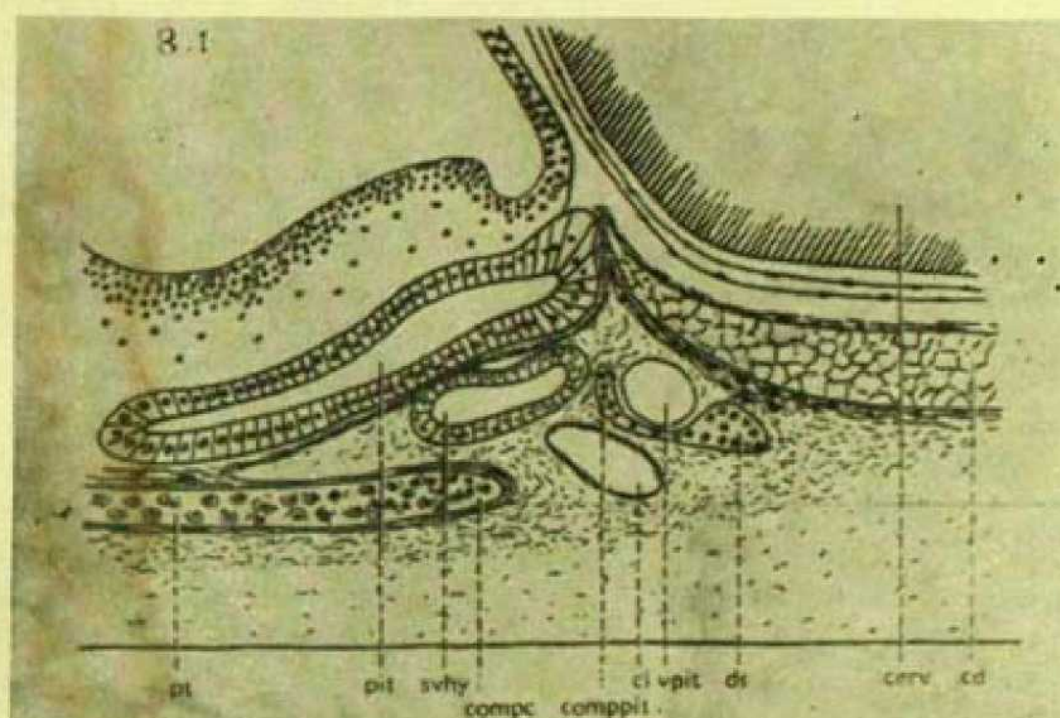


Fig. 8.1. *Scylliorhinus canicula*. Sagittal section of the pituitary region of an embryo (after de Beer). (cd), notochord; (cerv), brain; (ci), internal carotid artery; (Com pc), precarotid commissure; (Com p pit), postpituitary commissure; (ds), dorsum sellae; (pit), pituitary body; (pt), trabecular plate; (svhy), ventral sac of the hypophysis; (v pit), pituitary vein.—From Devillers (1958). Courtesy of Dr. P. P. Grasse and Masson et cie Editeurs, Paris.

Two main types of plagiostome pituitaries could be recognized by Stendell (1914) and Norris(1941). (1) The *squaloid type*: it is found in sharks, dogfishes and sharklike rays. The pars distalis is hollow. The head of the dorsal lobe contains vesicles or tubules which communicate with the spacious hypophysial cavity. The tail of the dorsal lobe (mesoadenohypophysis) consists of simple folds of the wall of the large hypophysial cavity. Norris(1941) observed in *Chlamydoselachus* that the intermedia also may consist of hollow tubules; but Meurling(1962) observed that the intermedia is usually composed of compact strings which tend to coalesce. (2) The *batoid type*: it is found in typical skates and rays. The hypophysial cavity is small or obliterated and the entire dorsal lobe



"is a more compact structure consisting of cords and clusters of cells" (Wingstrand, 1966). The dorsal lobe of the holocephalian is always hollow. The ventral lobe of the selachian is hollow and often vesicular (Ball and Baker, 1969). Holmes and Ball (1974) stated that these spaces and vesicles represent a persistent hypophysial cleft and the colloid within them is PAS-positive, aldehyde fuchsin-positive, and alcian blue-positive. The colloid may contain gonadotrophin and thyrotrophin. Della Corte (1961) and Mellinger (1962) found the glandular cells of the ventral lobe to be of one type and basophilic. There are indications from recent works that the colloid is secreted by nonendocrine pericavity cells. Mellinger (1969) and Alluchon-Gerard (1971) found these cells to be chromophobic and they were linked to each other by desmosomes and they secrete glycoproteinaceous colloid akin to that of the mucus secreting stomodeal epithelial cells. These cells may be homologous with the stellate cells found in the adenohypophysis of other vertebrates (Vila-Porcile, 1972). Variable number of giant cells have been noted in the walls of the selachian cavities and Mellinger (1969) thinks that the colloid may be resorbed by them.

van Oordt (1968) said that in the Pleuroremata and Hypotremata the proadenohypophysis has one cell type. These cells are elongated and the granules are strongly PAS-positive and lightly AF-positive. These granules can also be stained by erythrosin, acid fuchsin and orange G and they are rich in sulfhydryl groups. The mesoadenohypophysial cells are elongated and the granules varying in amounts are erythrosinophilic, orangeophilic and weakly PAS-positive. Cyanophilic granules are also present. Mellinger (1960, 1962, 1963, 1964, 1966) noted the presence of only one cell type and depending upon the functional state of the cells they contained either orangeophilic or cyanophilic granules. However, two cell types have been described in the mesoadenohypophysis by Della Corte (1961) and Della Corte and Chieffi (1961). One cell type is situated dorsally and it is acidophilic. More ventrally situated cells are cyanophilic. The metaadenohypophysial cells which line the sinusoids are ovoid or elongated. One pole extends towards the blood vessels. Centrally situated cells have no such protrusion. The cells contain faintly PAS-positive and weakly erythrosinophilic granules. Ventral lobe-cells are elongated and the granules are strongly PAS +, AF + and AB +. Similar strong reactions have been noted for the colloidal material situated in the cavities of the pars distalis and the ventral lobe. Basophils, acidophils and chromophobes are present in the dorsal lobe of Holocephalians. Two types of basophils have been noted in the Rachendachhypophyse. Both of them are PAS+; one type is AF+ while the other is AF- (Ball and Baker, 1969).

The pituitary of *Scyliorhinus caniculus* has been studied with electron microscope by Mellinger (1964) and the neurointermediate lobe of the same species has been investigated by Knowles (1965). Spherical secretory granules in the cells of the proadenohypophysis vary from 100 to 300 or 400 nm in diameter. Those in the mesoadenohypophysial cells vary from 100nm to 900nm in diameter. Two separate cell types have been detected in the ventral lobe. One group of cells has large granules of 400 to 1,000 nm in diameter. The other group



of cells has small granules of 70 to 100 nm in diameter. The metaadenohypophyseal cells have granules of 300 nm in diameter but sometimes very large granules (1,300 nm in diameter) are encountered in the basal part of the cells which line the blood vessels. These granules have an electron dense outer rim and the centre is electron—lucent. Alluchon-Gerard(1978) described the ultrastructure of the dogfish adenohypophysis. The ventral lobe is the major gonadotrophic part of the pituitary (Dodd *et al.*, 1960; Firth and Vollrath, 1973; Lance and Callard, 1978; and Sumpter *et al.*, 1978). Biochemical and autoradiographic studies on the estradiol—concentrating cells in the diencephalon and pituitary gland of the female dogfish (*Scyliorhinus canicula* L.) were conducted by Jenkins *et al.* (1980). The main areas of label concentration were the preoptic, habenular, hypothalamic tuberal nuclei, and the ependyma of the third cerebral ventricle, as revealed by the autoradiographic study. Estradiol uptake was very little in the pituitary lobes.

Immunochemical and biological studies with antiserum to shark growth hormone (GH) indicate that structure of shark GH differs significantly from that of modern bony fishes (Hayashida and Lewis, 1978).

Dodd(1960) and Mellinger(1962) noted hyperactivity of the cells in the ventral lobe of dogfishes (*Scyliorhinus caniculus*) with enlargement of thyroids. Their conclusion was that the thyrotrophs were situated in the ventral lobe of the adenohypophysis. Injections of the extracts of the same animal into the newly hatched dogfishes markedly increased radioactive iodine uptake. Extracts from other parts of the pituitary did not show the same result (Dent and Dodd, 1961). Thyrotrophic function was however ascribed to the proadenohypophysis by Della Corte and Chieffi(1961) as these cells differentiate early in the young *Torpedo marmorata*.

Surgical hypophysectomy either total or partial and replacement therapy with mammalian hormones proved that the ventral lobe secretes gonadotrophins in dogfish and Skate (Dodd,1960; Dodd *et al.*,1960). Mellinger(1964) also confirmed the conclusion that the cells of the ventral lobe have a gonadotrophic function. In female dogfishes there is considerable increment in the size of the ventral lobe at sexual maturity than the rest of the pituitary (Dodd,1960; Dodd *et al.*,1960; Della Corte,1961; Mellinger,1964). Della Corte and Chieffi(1961) noted a correlation between the cells of the ventral lobe and gestation in the female *Torpedo marmorata*. deRoos and deRoos(1967) obtained ACTH activity in the head of the dorsal lobe by bioassay. Sumpter *et al.*(1978) studied the purification and properties of gonadotrophin from ventral lobes of the pituitary gland of the dogfish. Sumpter *et al.*(1978) concluded that the main source of gonadotrophin is the ventral lobe of the dogfish pituitary. Other lobes contain small amounts of a similar, if not identical, hormone.

The *Rachendachhypophyse* of Holocephali consists of follicles and cysts with small and large chromophobes and also AF-positive cells (Sathyanesan,1965). *Hydrolagus coliei* pituitary has been studied by Sathyanesan(1965) and Jasinski and Gorbman(1966). Dodd *et al.*(1980) noted the presence of a gonadotrophin



in the rachendachhypophyse of the pituitary gland of the rabbit fish *Hydrolagus collieri* and it is the main source of gonadotrophin in the holocephalan pituitary and thus it appears to be the functional equivalent of the ventral lobe of the elasmobranch (from Ball and Batten, 1980).

*Pituitary of Hydrolagus collieri*

Part of the pituitary	Cell types
Proadenohypophysis	Fuchsinophilic, PAS-negative, AF-negative
Mesoadenohypophysis	PAS- and AF-positive basophils and acidophils
Metaadenohypophysis	Acidophilic cells

It has been suggested that the ventral lobe secretes gonadotrophin as has been detected by bioassay (Scanes *et al.*, 1972), total or partial hypophysectomy and replacement treatment with mammalian hormones.

Hormone	Location of cells	Method of study	Authors
Gonadotrophin	Ventral lobe	Bioassay	Scanes <i>et al.</i> (1972)
		Hypophysectomy (total or partial).	
		Replacement treatment with mammalian hormones.	see before
		Histology and ultra-structure of testis.	Dobson and Dodd (1977)
TSH	Ventral lobe	Bioassay	Ball and Baker (1969) for references. Mellinger (1972)
		Ultrastructure after thyroidectomy. Changes in cells with small granules (110nm).	Alluchon-Gerard (1978)



Hormone	Location of cells	Method of study	Authors
ACTH	Head of the dorsal lobe	Bioassay	deRoos and deRoos (1967)
		Chemical or surgical adrenalectomy	Mellinger (1969, 1972)
LTH	Head of the dorsal lobe	Bioassay	Sage and Bern(1972)
STH	Tail of the dorsal lobe?	.....	Holmes and Ball(1974)

Hoar(1969) discussed the reproduction in fishes. True viviparity has been found only in elasmobranchs and teleosts. In both the groups there are ovoviparous and viviparous species. In the former one there is plenty of yolk in the eggs for the nourishment of the young and only protection is afforded by the female. In the latter type the yolk is greatly reduced and the developing young is connected with the maternal tissues in very early stage and derives nourishment from the maternal tissues. Viviparity has been reviewed by Hoar(1957, 1969), Budker(1958), Bertin(1958) and Amoroso(1960). In Chondrichthyes the fertilization is always internal.

Budker(1958) divided the Chondrichthyes into oviparous and viviparous groups. The viviparous was subdivided into aplacentals and placentals. The maternal-foetal relationships in the elasmobranch fishes have been summarized by Ranzi(1934) (fig. 8.2). The rectangles on the lefthand side of the ordinate shows the amount of organic matter which is being derived from the mother by the embryo. (—) sign indicates that this does not happen. The rectangles to the righthand side of the ordinate shows the content of the organic matter in the uterine fluid. The dotted lines with (?) indicate probable values. Reduction of the maternal liver during development is measured by factor R and indicated by black bars. Histological structure of the uterine lining is shown on extreme righthand side.

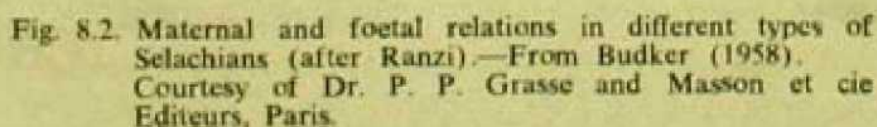
Budker(1958) described three types of uterine secretions: serous secretion (feeble), mucous secretion (intense), and lipidic secretion (abundant).

Figures 8.3 and 8.4 show the relations of maternal and foetal tissues and the placenta of *Mustelus canis* (after Ranzi).

In pregnant female selachians Ranzi(1936, 1937) noted hypertrophy and hyperaemia of the pituitary with decreased acidophilia. Chieffi(1961, 1967) and Della Corte and Chieffi(1961) confirmed these findings in elasmobranchs. Hoar



Hypophysectomy in *Mollienesia latipinna* during pregnancy did not show any bad influence (Ball, 1962). The reverse effect was, however, noted by Chambolle(1964) in *Gambusia*.



*Pars intermedia*: In selachians it secretes MSH. Mellinger (1963) found hyperplasia and degranulation of the cells of this location in *Scyliorhinus caniculus* after lesions of the hypothalamus or neurohypophysis. An increase in the amount of ergastoplasm was observed in these cells. The melanin granules



dispersed in the melanophores of the skin of such animals. Similar effects were observed after heterotopic transplantation of the neurointermediate lobe.

The pars intermedia cells of the elasmobranch contain osmiophilic and acidophilic globules. Della Corte(1961), Meurling(1963) and Mellinger(1963) thought that these globules are due to cellular degeneration. Knowles(1965) considered them to be hormone stores.

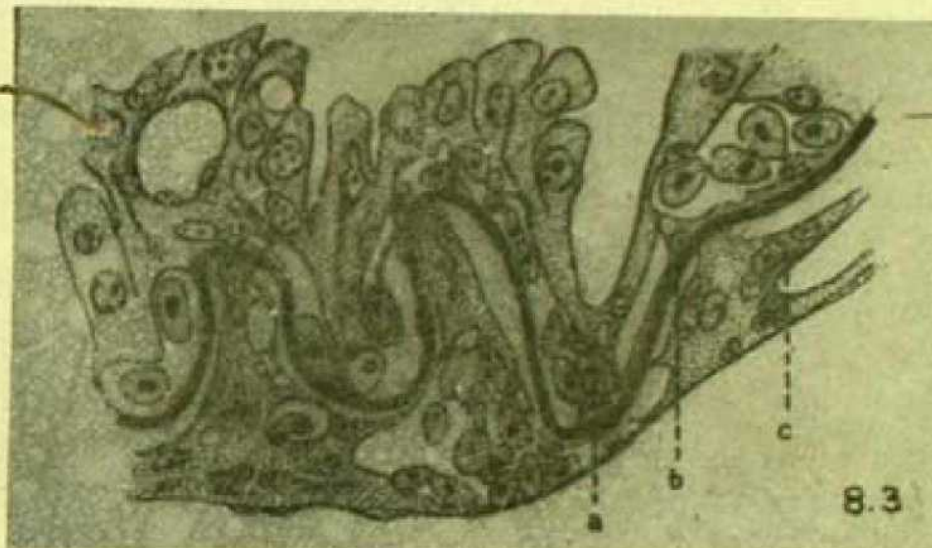


Fig. 8.3. Embryo of *Mustelus canis*. Placental region. (a), foetal tissue; (b), envelop of the egg; (c), maternal tissue (after Ranzi).—From Budker (1958). Courtesy of Dr. P. P. Grasse and Masson et cie Editeurs, Paris.

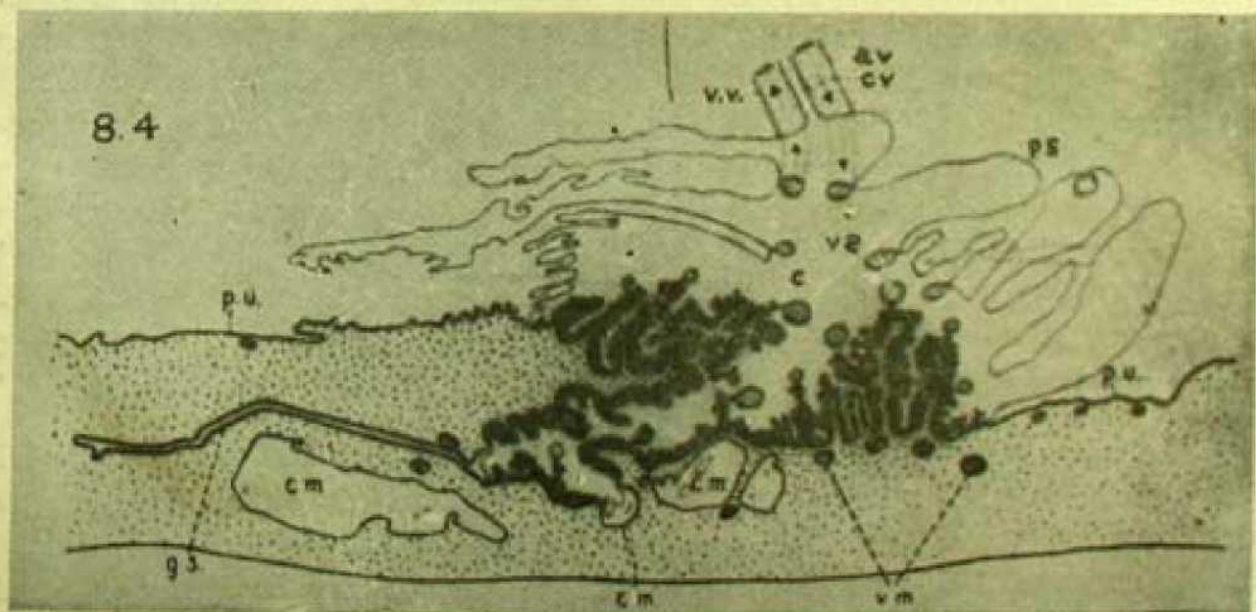


Fig. 8.4. Placenta of *Mustelus canis*. The uterine wall and the maternal tissues are in black below; above there are foetal tissues. (av), umbilical artery; (ps), Yolk sac wall; (c), Yolk sac cavity; (cm), mucous glands; (cv), vitellointestinal duct; (gs), sacciform glands; (pu), wall of the uterus; (ve), foetal blood vessels; (vm), maternal blood vessels; (vv), umbilical vein (after Ranzi).—From Budker (1958). Courtesy of Dr. P. P. Grasse and Masson et cie Editeurs, Paris.



Lobular arrangement of the intermedia cells is present and the lobules are separated by highly vascular connective tissue or there is a fusion of the cell cords to form a mass penetrated by blood vessels (Meurling, 1972). Penetration of neurohypophysial fibres may be less in the sharks or more extensive as in the dogfish and the rays.

Cells in the intermedia of *Scyliorhinus stellaris* were differentiated into central and peripheral cells by Knowles (1965) depending on the shape, situation and ultrastructure. The peripheral cells lying close to the blood vessels act as store house of MSH and sudden release of the hormone can take place. MSH is not stored in the central cells and a slow and steady release of the hormone from these cells may occur. The peripheral cells have a synthetic apical region and a basal region for hormone release. Type A or peptidergic fibres make direct synaptoid contacts with the synthetic poles of the intrinsic intermedia cells and type B or aminergic fibres make similar contacts with the release poles. Knowles *et al.* (1970) found greater complexity of innervation. Type A and type B fibres innervate the synthetic pole in *S. stellaris*. Meurling and Björklund (1970) observed that in the Skate *Raja radiata* both types of fibres innervate both the poles of the intermedia cells. These nerve fibres also innervate the blood vessels in addition to the innervation of the cells. Mellinger (1962, 1963, 1964) observed only type A fibres in the neurointermediate lobe of *S. caniculus*. Meurling (1972) thought that the inhibitory control of MSH secretion involves catecholamines in the intermedia and it operates through neurovascular and neuroglandular mechanisms. Chevins and Dodd (1970) ascribed the inhibitory control to the preoptic nucleus by lesion of the nucleus, section of the tract and ectopic transplantation of the neurointermediate lobe in *Raja* species. In *Raja* synthesis inhibition of and release of MSH is through type B aminergic fibres (Meurling *et al.*, 1969; Meurling and Björklund, 1970) and type A fibres do not control the cellular function. Holmes and Ball (1974) stated, "to reconcile all these findings, one must postulate either marked species variation, even within the same genus, in the role played by type A and type B fibres, or else suppose that the inhibitory type B fibres in the species studied by Chevins and Dodd (1970) originate in the preoptic nucleus rather than in the nucleus lateralis tuberis. The matter clearly requires further investigations, although the salient feature of a hypothalamic inhibitory control of MSH secretion is firmly established."

**The neurohypophysis:** The saccus vasculosus is developed from the dorsal wall and the lateral diverticuli of the embryonic saccus infundibuli. The saccus vasculosus has typical crown cells (Dammerman, 1910; Tilney, 1915; v.d. Kamer and Schuurmans, 1953; and Dohrn, 1955). These cells may be absent in some specimens of *Dasyatis* and *Torpedo* (Bargmann, 1954). The ventral wall of the embryonic saccus infundibuli gives rise to the neurohypophysis which is situated ventral or caudal to the saccus vasculosus. The neurohypophysis has a thin wall which is in contact with the intermedia and the processes from the former proceed into the latter. The preoptic nucleus sends fibres into the preoptico-neurohypophysial tract. The neurosecretory material (nsm) is AF-positive which



can be seen in the projection of the dendrites into the third ventricle. Some of the fibres in the tract end on the primary portal capillaries in the median eminence (Perks, 1969). Granules of 180nm in diameter in type A1 fibres are destined for the neurointermediate lobe and granules of 130nm in diameter in type A2 fibres are for the median eminence (Mellinger, 1964). The granules in the nucleus lateralis tuberis are AF-negative, and belong to type B granules and are 80nm in diameter (Mellinger, 1962). The fibres proceed to the median eminence and the neurointermediate lobe. Urano (1971) noted monoamine oxidase in these cells. Similar activity has been observed around the blood vessels of the neurointermediate lobe of the dogfish *Triakis*. In the neurointermedia fibres of the preoptic nucleus are very near the ependymal lining of the infundibular recess and the ependymal processes are directed ventrally and are noted to mix with the neurosecretory fibres. Wingstrand (1966) said, "The processes into the intermedia may be short and broad and tend to interdigitate with the cell cords of the intermedia as in squalus, or the limiting membranes between the lobes may have disappeared and the nerve fibres spread into the intermedia in a diffuse manner (Raja) or in bundles, which enter the cell cords as in *Scyllium* (Meurling, 1962). The neurohypophysial processes are hollow in Hexanchoid sharks and *Chlamydoselachus* (Stendell, 1914; Norris, 1941), partly also in *Squalus* (Meurling, 1962)."

*Median eminence*: Meurling (1960) and Mellinger (1960) described the median eminence in sharks and rays. Its location is in between the lobi inferiores of the brain and it is in close contact with the anterior end of the adeno-hypophysis. The superficial neurosecretory nerve endings are in the palisade vessels pass into the pars distalis in *Squalus*. In *Raja* and *Scyllium* portal layer having an intimate contact with a dense capillary net. Few small portal vessels also pass to the neurointermedia. The elongated median eminence of the Holocephalian is not divisible into anterior and posterior parts.

A connective tissue sheet separates the median eminence from the dorsal lobe of the pituitary and Mellinger (1966) found it to be missing at places. The median eminence has an anterior and a posterior part, corresponding to the head and tail of the dorsal lobe of selachians. The primary capillary plexus is formed by the branches of the inferior hypothalamic artery (Mellinger, 1964; Follenius, 1965; Jasinski, 1969). In the posterior part of the median eminence of the dogfish the primary capillaries form two groups of glomerular vessels which are longitudinally disposed and the glomerular loops penetrate upwards into the palisade layer. In the anterior part of the median eminence the primary capillaries are continuous with the capillaries of the head of the dorsal lobe, there being no portal vessels. The glomeruloid capillaries in the posterior median eminence proceed as portal veins and communicate with the capillaries of the tail of the dorsal lobe. Preoptic nucleus sends type A2 fibres with secretory granules of 130nm in diameter and nucleus lateralis tuberis sends type B fibres with granules of 80nm in diameter to the primary capillaries of the anterior part. They (both types) also end on the glomerular vessels of



the posterior median eminence (Mellinger, 1960, 1962, 1964, 1966; Mellinger *et al.*, 1962; Follenius, 1965; and Chevins, 1968). The ventral lobe of the pituitary does not get any portal blood but Chevins(1968) found it in Raja. There is no direct neurosecretory innervation of the dorsal and ventral lobes (Holmes and Ball, 1974).

Gonadotrophic(LH-like) cells have been noted in the ventral lobe by immunofluorescence and ACTH cells have been located in the head of the dorsal lobe of selachians (Mellinger, 1972) and also occasionally in the neurointermediate lobe. Fontaine and Oliverreau(1975) said "However, there is no cross-reaction with anti- $\alpha$ MSH and anti- $\beta$ MSH antibodies, or with porcine ~~anti~~-ACTH and porcine anti- $\beta$  and  $\alpha$ LPH (lipotropic hormone) (Mellinger and Dubois, 1973)".  $^{32}\text{P}$  uptake by the young chick gonad, influenced by TSH and LH shows gonadotrophic activity only in the ventral lobe (Scanes *et al.*, 1972).

The ependyma of the neural lobe is well developed in most of the non-mammalian species, especially in fishes, reptiles and birds. In the non-mammalian species an anatomical link is formed by the ependymal cells between the cerebrospinal fluid and the neural lobe. An inverse numerical relationship exists between the ependymal cells and pituicytes. With few exceptions, no pituicyte is found in the neural lobe of most non-mammalian species. In most mammalian species the ependyma is apparently absent and plenty of pituicytes in the neural lobe could be found.

Regarding the blood supply of the neurointermediate lobe some important species differences do exist (Rodriguez, 1971).

A second hypophysiportal system was described by Mellinger(1960) in *Scyliorhinus caniculus*. Large areas of diencephalon and mesencephalon drained by this portal system and the collecting veins branch in the neurointermediate lobe. Meurling(1967) found similar arrangement in four other elasmobranch species. The degree of branching of the portal (afferent) veins in the neurointermediate lobe is variable. It is maximally noted in *Scyliorhinus* and *Pristiurus*, and least noted in *Etmopterus*. Only the dorsal lobe in Holocephalians receives portal blood and no portal supply to the neuro-intermedia is found (Jasinski and Gorbman, 1966; Meurling, 1967).



## CHAPTER 9

### THE PITUITARY OF THE PRIMITIVE ACTINOPTERYGIAN FISHES

The typical characteristics of the actinopterygian pituitary according to van Cerd (1968) are :

- (a) the lateral lobes are completely absent as also the pars tuberalis developing from these ;
- (b) the cells in the pars distalis are segregated and thus that part of the adenohypophysis is divided into a rostral and a caudal zone ;
- (c) the contact between the pars nervosa of the neurohypophysis and the pars intermedia of the adenohypophysis is intimate ;
- (d) a direct innervation of the pars distalis from the more rostral part of the neurohypophysis, i.e. the median eminence occurs.

#### *The pituitary of super-order Chondrostei*

##### *1. Order Brachiopterygii and Polypteriformes*

The base of Rathke's pouch was seen in the adult as a duct to the oral roof from an adenohypophysial cavity in the *Polypterus* and *Calamoichthys*. Solid ramifications of the neurohypophysis invaded the posterior part of the adenohypophysis. Dohrn (1955) could not find saccus vasculosus in *Calamoichthys*. Wingstrand (1966) described the pituitary of *Polypterus*. Three histological parts could be identified in the adenohypophysis. The proadenohypophysis is an elongated structure situated anteroventrally in the gland having weakly staining (AF) cells. This part of the gland was continuous anteriorly with a thick vascularized ligament attached to the preadenohypophysial brain wall. The irregular cavity in the proadenohypophysis is lined with a continuous layer of tall prismatic cells. The mesoadenohypophysis contains both light and dark cells (AF stain). Small, little stainable cells of the metaadenohypophysis surround the ramifications of the neurohypophysis. Saccus vasculosus had crown cells and the neurohypophysis had plenty of neurosecretory fibres. A typical median eminence could be found which was in contact with the vascularized ligament. A typical palisade layer outside the thick bundles of neurosecretory fibres was present. These were close to the capillaries which communicate with the vessels in the vascular ligament.

Kerr (1968) noted that the lining epithelial cells of the hypophysial duct had strongly alcian blue positive and weakly PAS-positive secretory material. These were similar to the cells which lined the roof of the mouth. The portal vessels enter the rostral tip of the pars distalis. In *Polypterus* and *Calamoichthys*, fluore-



scent antibody to ovine LTH was bound by cells which were found throughout the pars distalis (Aler, 1971). The duct cells are therefore not lactotrophs. Kerr (1968) said that the proadenohypophysis contains only one type of basophil cells which are strongly PAS-positive and weakly AB-positive. In the mesoadenohypophysis some of these cells have been noted. Lagios (1968) found two types of basophils in the proadenohypophysis. In the modern histological description of the pituitary of *Polypterus senegalus*, *P. bichir* and *Calamoichthys calabricus* Kerr (1968) remarked that the proadenohypophysis is formed by one cell type only, apart from the cells of the duct. van Oordt (1968) said that the strongly PAS+ and weakly AB+ cell type of Kerr "is not much different from the basophils type 3 of amphibians and dipnoans, both in stainability of the secretory granules and in distribution. If this is so, it can be said that the proadenohypophysis of the polypterine pituitary is homologous with the zona tuberalis (Wingstrand, 1966) of the amphibian and dipnoan pars distalis. Not only do both develop from the same most rostral tip of the pars distalis, but there is also a common relation with the portal vessels and a great similarity in cellular composition. This does not mean, of course, that the cells in the proadenohypophysis of the polypterines have the same function as those in the tuberal zone of amphibians".

Kerr (1968) observed one type of acidophil and three types of basophils in the mesoadenohypophysis. The acidophil cells are small and have orange G-positive and erythrosine-positive granules. The basophil cells of the predominant cell type are of moderate size, elongated and are full of granules which are strongly PAS-positive and AB-positive. The cells of the second basophil cell type are degranulated and weakly stained by PAS and AB but AF-positive even without previous oxidation. The third type of basophil cells is same as is noted in the proadenohypophysis. The cells in this location were not arranged in zonal pattern. Hayashida (1971) noted STH in *Polypterus* pituitary which was immunochemically and biologically similar to rat STH.

Only one cell type was present in the pars intermedia. This is a small basophilic cell and the granules were PAS-positive, aniline blue-positive and lead haematoxylin-negative. The cells are arranged radially surrounding the neurohypophysial projections. They form a true neurointermediate lobe (Kerr, 1968). Neurosecretory fibres did not penetrate into the pars distalis.

No nucleus lateralis tuberis has been specially noted in the hypothalamus but the median eminence contains type B fibres along with type A fibres from preoptic nucleus which is aldehyde fuchsin positive. There is possibility for type B fibres to come from the preoptic nucleus if the nucleus lateralis tuberis is absent. The preopticoneurohypophysial tract ends in the neural lobe. Type A fibres contain granules which are electron-lucent and 106 to 167nm in diameter. Lagios (1968) found that these fibres end synaptically on glial processes which surround the perivascular space of the neural lobe capillaries. Below the ependyma the median eminence has got aldehyde fuchsin-positive preopticoneurohypophysial tract and on the outer aspect of this layer a thick palisade layer is present having



little or no aldehyde fuchsin-positive neurosecretory material. The nerve fibres around the primary portal vessels are mainly type B fibres and the electron-dense granules are 91 to 106nm in diameter. Synaptic vesicles can also be detected. The fibre endings make synaptic contact with the basement membrane of the perivascular space. Fenestrations in the capillary endothelium and pinocytotic vesicles have also been noted by Lagios(1968).

Franzoni *et al.*(1978) described the hypothalamo-hypophysial system in *Calamoichthys calabaricus smithi* (Polypteriformes).

The typically stratified median eminence contains three different types of nerve fibres and terminals having: (1) granular vesicles 600-800 Å in diameter and clear vesicles of 300-450 Å; (2) granular vesicles (900-1200 Å), polymorphous dense granules (1300-1600 Å), and clear vesicles (300-450 Å); (3) dense granules (1600-1800 Å) and clear vesicles (300-450 Å). There are synaptoid contacts between nerve fibres of different types and ependymal-glia cells, active points between nerve fibres and the perivascular space, and typical synapses between nerve fibres.

In the pars nervosa of *Calamoichthys* aldehyde-thionine + nerve fibres contain elementary neurosecretory granules (1000-1500 Å), and larger neurosecretory granules (1800-2000 Å) are sometimes met with. Some pituicytes are found. Ependymal cells line the infundibular lumen.

Aldehyde-thionine + bipolar cells with CSF-contacting processes are present in the perinfundibular grey of the caudal hypothalamus, near the median eminence. The cells have elementary neurosecretory granules (1600-2000 Å) or dense granules (1100-1800 Å), granular vesicles (750-1000 Å) and multivesicular bodies.

The authors suggest, "At least some of the afferent fibres to the median eminence are likely to be supplied by the region that passes almost indistinctly into the median eminence, where some neurosecretory neurons are still found".

## II. Order Acipenseroides (sturgeon)

Tilney(1912), Stendell(1913, 1914), Kerr(1949) and Polenov and Barannikova(1958) studied the pituitary of sturgeons (*Chondrostei*). Barannikova(1949, 1950) and Ivanova(1953) discussed the histophysiology. No epithelial stalk is present in the sturgeons. A large hypophysial cavity is found between the pars distalis and the pars intermedia. The mesoadenohypophysis is small and situated middorsally and it is composed of tubules which open into the hypophysial cavity. In the posterior part of the proadenohypophysis such small tubules were found. Wingstrand(1966) said, "the anterior end of this lobe contains closed acini or epithelial vesicles as in primitive teleosts." Plenty of hollow diverticuli of the neural lobe penetrates into the intermedia as is found in lungfishes but the intermedia has not got a tubular structure. Wingstrand(1966) further stated, "embryological investigations indicate that the hypophysial cleft



is formed as a schizocoel in an originally compact anlage but do not allow any further comparisons with other vertebrates."

Most of the cells of the rostral pars distalis are situated around small vesicles and most of them are acidophilic. Basophils and chromophobes are less in number. Sage and Bern(1972) noted that the acidophils of rostral pars distalis may secrete LTH and this has been found in *Acipenser* pituitaries by bioassay. Hansen and Hansen(1975) localized growth hormone and prolactin immunohistochemically in the pituitary gland of *Acipenser guldenstaedti* Brandt.

van Oordt(1968) mentioned that the mesoadenohypophysis contains acidophils, basophils and chromophobes. In *Acipenser* these cells line the vertical tubules which communicate with the hypophysial cavity. This cavity separates the meso from the metaadenohypophysis. Hayashida and Lagios(1969) could attach STH secretion to the acidophils of this zone by bioassay and radioimmunoassay in the acipenseroid pituitary. Holocrine extrusion in ventral basophils of the pars distalis was observed by Barannikova(1964) at the starting and progress of the spawning period of *Acipenser guldenstadti*. These basophils completely disappeared after oviposition and the gonadotrophin content of the pituitary followed the same pattern. The ventral basophils were thought to be the source of gonadotrophin secretion (Barannikova, 1964). In the sturgeon thyrotrophic(Jakovleva, 1970), gonadotrophic (Barannikova, 1969) and rostral corticotrophic function(Barannikova, 1974) could be detected.

Nerve fibres from the floor of the infundibulum were found to pass to the pars distalis of *Amia* occasionally but such fibres could not be demonstrated in the *Lepisosteus* and *Acipenser*.

A quantitative study was done by Polenov and Pavlovic(1978) regarding the functional morphology of the peptidergic neurosecretory cells in the preoptic nucleus of the sturgeon, *Acipenser guldenstadti* Brandt.

The intermedia has plenty of basophilic cells and some acidophils and chromophobes are also present. It is penetrated by branched processes of neural lobe.

Sathyanesan and Chavin(1967) found the aldehyde fuchsin-positive preoptico-neurohypophysial fibres to proceed to the neural lobe from the preoptic nucleus. Most of the cells of the nucleus lateralis tuberis are aldehyde fuchsin-negative but a few cells are AF-positive.

Ependymal cells, pituicytes, and type A and type B fibres are present in the neural lobe. Polenov *et al.*(1972) found catecholamines in type B fibres. Two types of secretory granules are contained in type A fibres. One type of granule is 140 to 180nm in diameter and the other 100 to 140nm in diameter. The type A fibres start from the preoptic nucleus. The secretory granules of type B fibres are 40-110 nm in diameter and they start from the nucleus lateralis tuberis. Some of these fibres have contacts with ependymal cells in the neural lobe.



The median eminence and the portal system are well developed (Hayashida and Lagios, 1969; Ball and Baker, 1969). The pars distalis does not get any neurosecretory innervation. Between the anterior neurohypophysis and the pars distalis there is a vascular connective tissue sheet and the neurosecretory fibres from the preoptic nucleus terminate around the capillaries and this site of contact may function as a median eminence (Sathyanesan and Chavin, 1967; Kerr, 1968; Polenov, 1966). Hayashida and Lagios (1969) said that at the proximal contact area only type B fibres are met with. These type B fibre endings contain secretory granules of 76 to 105nm in diameter. The preopticoneurohypophysial tract contains granules of 130nm or more in diameter and the tract passes to the neural lobe. The type B fibre endings appose against basement membrane of the collagenous pericapillary space after mixing with glial processes. The capillary endothelium has fenestrations opposite the fibre endings. Synaptoid regions of neurosecretory axon terminations are also noted. The source of the type B fibres is the nucleus lateralis tuberculi.

### *The pituitary of super-order Holostei*

Wingstrand (1966) said that the pituitary of these fishes is very similar to that of the teleosts. The proadenohypophysis is vesiculate like the primitive (isospindylous) teleosts and no open duct communicates with the oral cavity. "The saccus vasculosus is large, and the neurohypophysis invades the metaadenohypophysis with hollow processes, the lumen of which communicates with the ventricle." The development of the holostean adenohypophysis is more or less similar to that of the teleosts. The schizocoelic cavity appears in the compact anlage of the adenohypophysis of *Amia* but in the adult, the cavity is reduced (Smith, 1914; de Beer, 1923).

Ball and Baker (1969) studied the pituitary of Holostei in greater details using the different staining techniques. In *Amia* and *Lepisosteus* no hypophysial cavity or duct is present in the adult. The pituitary gland is attached to the infundibular floor (anterior neurohypophysis) along the whole length. The pituitary in *Amia* is relatively shorter and deeper than in *Lepisosteus*. The saccus vasculosus is large. *Amia*-pituitary is described below after Ball and Baker (1969) and Holmes and Ball (1974). There are closed vesicles or follicles in the rostral pars distalis. Kerr (1949) thought that these follicles represent diverticuli from the hypophysial cleft. The follicles are lined by tall acidophils which are erythrosinophilic after Herlant's Alizarin blue tetrachrome (Aliz BT). These are probably the source of LTH (Sage and Bern, 1972). They are *acidophil 1*. Amongst these cells a second cell type is present which is *acidophil 2* and they do not reach the follicular lumen. The cells are erythrosinophilic but they also take alizarin blue so that they are prominently blue-red in contrast to the scarlet acidophil after Aliz BT. These cells are strongly lead haematoxylin positive and they were thought by the authors to resemble the teleostean corticotroph. The colloid material within the follicular lumen is PAS +, AB + and AF +. Ventrally *basophil 1* cells have been detected. These cells are PAS +, AF +, AB +,



and PbH +. The authors state, "However, after Aliz BT, its coarse refractile granulation is brilliantly stained with erythrosin; the cell usually also contains a few large perinuclear aniline blue+ granules, which, together with the larger size of its erythrosinophilic granules and details of cell shape, allow an easy distinction between basophil 1 and the erythrosinophilic acidophil 1 after Aliz BT. It may be that basophil 1 should be called an amphiphil." These cells form a mantle which covers the ventral and lateral parts of the gland and encloses the proximal pars distalis and neuro-intermedia and have a mixture of acidophils 1 and 3. Basophil 1 may be a gonadotroph.

In the proximal pars distalis vertical cell cords can be found and prominent cell type is *acidophil 3*. This cell class can be differentiated from acidophils 1 and 2 by staining with orange G after Aliz BT. Acidophil 3 is possibly a somatotroph. It is slightly PAS+. Hayashida(1971) found STH in *Amia* and *Lepisosteus* having biological and immunochemical resemblance to mammalian STH. Plenty of acidophil 3 cells are situated dorsally near the median eminence and infundibular floor and this cell type is found also in the proximal pars distalis.

*Basophil 2* is small and of rounded shape. It is clearly blue in Aliz BT and slate-blue after AB-PAS-OG and AF+ and PbH+. It occupies the dorsal and central part.

*Basophil 3* is larger and of angular shape. It stains lavender in Aliz BT with scattered red granules and also dull blue granules and a few large clear blue granules are present near the nucleus. With AB-PAS-OG it stains magenta and is more strongly AF+ and PbH+ than basophil 2. Basophil 3 is situated in ventral and lateral parts. Holmes and Ball(1974) said, "Basophils 2 and 3 probably correspond to the two basophils described by Kerr(1949), and one of them is presumably a thyrotroph". The function of the other cell type is uncertain at present.

The branching processes of the neural lobe penetrate into the pars intermedia. These processes are hollow proximally and there is a lining of ependymal cells. The intermedia has two cell types. The club-shaped PbH+ cell is most frequent and the round, weakly PAS-positive cell is also met with. After Aliz BT, the PbH+ cells stain blue and the PAS+ cell is amphiphilic having red to mauve colour.

The preoptic nucleus can be divided into dorsal pars magnocellularis and ventral pars parvocellularis. The nucleus lateralis tuberis can be divided into several parts (Sathyanesan and Chavin,1967). The neurosecretory material in the neural lobe is AF+, AB+, PAS+, CAH+, and aniline blue+. The neurosecretory fibres come from the preoptic nucleus and Sathyanesan and Chavin(1967) found some AF—negative fibres from nucleus lateralis tuberis to end in the neural lobe. Lagios(1970) found that at the posterior cell cords of the pars distalis, the neural tissue is separated from the pars distalis cells by extravascular space, on the neural border of which can be found the endings of many type A fibres with granules from 124-201nm in diameter and also occasional type B



fibres with granules from 74-120nm in diameter. Synaptoid contacts between typeA fibres and intermedia cells have also been noted. Majority of such fibres make synaptoid contacts on the basement membrane of the collagenous intervascular space in between the neural processes and intermedia cells.

The median eminence and portal system of *Amia* was described by Ball and Baker(1969) and Lagios(1970). It is well developed and its blood supply is through a branch of the hypothalamic artery. Convolute preportal arterioles are also present. At the depth of the median eminence there are *glomeruloids* of two or three capillary loops with anastomosis between afferent and efferent limbs. They are similar to those noted in mammals and like the glomeruloids of the posterior median eminence found in selachians. Short portal vessels pass into the pars distalis from the glomeruloids. The anteriorly situated glomeruloids have endings of neurosecretory fibres on them with nsm as noted in nucleus lateralis tuberis (Ball and Baker,1969). Lagios(1970) found perivascular space to surround the glomeruloid capillary. Glial processes and plenty of synaptoid endings of typeB fibres abutting against the perivascular space are noted. Fenestration in the endothelium of the perivascular space has been noted. The space contains collagen fibres and several overlapping strata of endothelial cell processes. The anterior short portal vessels form secondary perifollicular capillary plexus in the rostral pars distalis and supplies the proximal pars distalis. Direct supply to the proximal pars distalis comes from the portal vessels which start from the more posteriorly situated median eminence. Convolute plexus intermedius is being supplied by capillaries from the pars distalis system.



## CHAPTER 10

### THE PITUITARY OF TELEOSTS

*The hypothalamus of the teleosts* (Kuhlenbeck, 1977) is extensive and can be divided into rostral preoptic hypothalamus and postoptic posterior hypothalamus, each of which can be subdivided into a dorsal (superior) subdivision and a ventral (inferior) subdivision. The following description is after Kuhlenbeck (1977).

The massa cellularis reuniens pars inferior is formed by the dorsolateral expansion of the periventricular elements and it extends into the telencephalon. Caudobasally the pars inferior blends with the entopeduncular cell groups which can be divided into suprapeduncular and interstitial nucleus of the basal forebrain bundles. The lateral subdivision of the pars preoptica hypothalami is represented by the rostral entopeduncular group, and the massa cellularis reuniens forms a transition between lateral and medial subdivision.

The preoptic recess is situated ventral to the anterior commissure. The medially situated periventricular cell mass of the pars preoptica hypothalami proceeds towards the rostrally located preoptic recess. *Neurosecretory magnocellular preoptic nucleus* and *nonneurosecretory parvocellular part* can either be separately discerned or they may be mixed up. The magnocellular nucleus is frequently highly developed in the form of discrete nuclear masses (Crosby and Showers, 1969). It may have a more rostral, an intermediate or a relatively caudal position. The parvocellular preoptic nucleus is disposed as dorsoventrally arranged rows of cells (*laminated appearance*) and may be condensed into *suprachiasmatic nucleus*, *diffuse supraoptic nucleus* etc.

More differentiation and complexity have been observed in the *posterior hypothalamic area* of Teleosts.

The postoptic posterior hypothalamus of teleosts has an ependymal organ of Kappers-Charlton and this is one of the circumventricular organs. The posterior hypothalamus has a superior and an inferior subdivision. The cell population comprises periventricularly situated cells and peripherally located cell groups (nuclei). Its anterior portion (*nucleus paraventricularis*) is continuous with periventricular preoptic cell masses.

The nucleus rotundus complex is situated in the dorsolateral posterior hypothalamus which has been considered by some as the Thalamus but others are of different opinion. It is also called by corpus glomerulosum pars rotunda (glomerular synapses) and consists of a dense neuropil and small and medium sized cells. They form a dense covering capsule. This complex merges into nucleus prerotundus, nucleus subrotundus, nucleus suprarotundus and nucleus posterior hypothalami.



The entopeduncular nucleus is scattered amidst the posterior part of basal forebrain bundles.

The inferior subdivision of the posterior hypothalamus contains lobi inferiores and saccus vasculosus. The lobi inferiores is divisible into medial (median) lobe, lateral lobe and posterior lobe and form the inferior part of the posterior hypothalamus. Several recesses are found in them. The saccus vasculosus is situated in the ventral part of the posterior hypothalamus. The anterior part of the lobus medianus contains paraventricular nucleus and nucleus tuberis anterior. These neurosecretory cells have relationship to the *hypophysennae* neurosecretory system of Diepen. The paraventricular nucleus includes the nucleus tuberis ventralis of Sheldon, the anterior hypothalamic nucleus and the ventromedial hypothalamic nucleus of Crosby and Showers (1969). The lobi inferiores contain the periventricular cells, nucleus diffusus lobi lateralis, nucleus tuberis lateralis, nucleus tuberis posterior (=nucleus sacci vasculosi), and nucleus mammillaris. The lateral hypothalamic nucleus (nucleus tuberis lateralis) can be regarded as merely a ventrocaudal extension of the nucleus preopticus magnocellularis (Kappers, Huber and Crosby; 1967). Kühlenbeck (1977) considered Sheldon's nucleus cerebellus hypothalami as a part of nucleus diffusus lobi lateralis. The ill-defined nucleus sacci vasculosi may be represented by hypothalamic cell groups in the posterior lobe of lobi inferiores. This nuclear group is situated dorsal to the saccus vasculosus and ventral to the tegmental cell cord of tuberculum posterius.

*Fibre connections (After Kappers, Huber and Crosby; 1967) :*

Telencephalo-hypothalamic paths in the cod is constituted by three descending tracts. They are the tractus olfacto-hypothalamicus medialis, the tractus olfacto-hypothalamicus lateralis and the tractus strio-thalamicus et hypothalamicus. The first mentioned tract is connected with the ventromedial or septal portion, the second connects with the lateral segment of the telencephalon, and the third connects with the striatal and epistriatal centres. Of the three, the first tractus is the smallest, myelinated and runs ventral to the tractus olfacto-hypothalamicus lateralis. It starts from the pars supracommissuralis septi and end in the lobi inferiores in front of the termination of the tractus olfacto-hypothalamicus lateralis. The authors thought them to represent the *septal portion of the fornix system* as found in higher vertebrates. In many teleosts this tractus is accompanied by direct olfactory fibres originating in the formatio bulbaris. The medial olfacto-hypothalamic tract is thought to have association with the tractus hypothalamo-olfactorius medialis. These two bundles form the *medial forebrain bundle*. The ascending tract starts from the nucleus tuberis posterior. Decussation of part of its fibres is obtained in the diencephalon and others in the anterior commissure.

The lateral olfacto-hypothalamic and strio-hypothalamic tracts contain both descending and ascending fibres and course jointly. "The descending fibres originate in the paleopallium, the epistriatum, and the striatum, and end, after a partial decussation in the commissura anterior, the nucleus entopeduncularis, the corpus



glomerulosum pars rotunda, and the most caudal part of the hypothalamus, the tuber posterior, where they come into synaptic relation with cells of origin of the medial longitudinal fasciculus, through which bundle impulses from the olfactory (and possibly also non-olfactory) centres of the telencephalon are transmitted to the efferent centres of the medulla oblongata and the spinal cord. Similar hypothalamic connections are maintained by the basal olfactory tract of higher vertebrates".

Dorsoventral and ventrodorsal fibres can also be found which connect the ventral thalamic and hypothalamic areas with the dorsal thalamus and the mid-brain (tractus thalamo-lobaris or mamillaris, tractus mesencephalo-lobaris or lobo-mesencephalicus and the tractus lobo-tectalis). The ascending fibres of the tractus thalamo-lobaris or mamillaris start from the large cells of the mamillary recess and lobi inferiores hypothalamici. They have been considered by some to be the equivalent of the *mamillo-thalamic* or *Vicq d'Azyr bundle* of mammals.

The tractus tubero-mesencephalicus starts from or ends at the tuber cinereum (anteromedial part of the hypothalamus) near the ventricle and courses dorso-laterally and terminate in the eminentia thalami or nucleus lentiformis.

Another connection between thalamic and hypothalamic areas is by the tractus geniculo-hypothalamicus of Franz.

Internuncial fibres connect different parts of preoptic and hypothalamic areas. Fibres start from preoptic nuclei and proceed to the tuber cinereum region (tractus prethalamo-hypothalamicus). They are unmyelinated. Posteriorly this tract is joined by fibres from the ventral hypothalamic nuclei and passes into the hypophysis and the saccus. Tractus intralobaris connects the tuber cinereum and the posterior part of the lobi inferiores. Tractus rotundo-lobaris or lobo-rotundus connects the hypothalamic with the ventral thalamic centres. The interrelating *commissural systems* are well developed.

### *The pituitary of Teleosts*

The pituitary of the teleosts has been reviewed recently by Green(1951), Herlant(1954), Pickford and Atz(1957), Dodd and Kerr(1963), Olivereau(1963), Stahl(1963), Olivereau and Ball(1964), Wingstrand(1966), Ball and Baker(1969), van Oordt(1968), Holmes and Ball(1974) and Jorgensen(1976).

The compact adenohypophysis of the adult teleosts consists of pro, meso, and metaadenohypophysis (Pickford). Olivereau divided the adenohypophysis into rostral pars distalis, proximal pars distalis and pars intermedia. The two former zones constitute pars distalis. The neurohypophysis consists of solid branching processes of nervous tissue projecting into the adenohypophysis (fig. 10.1). In the *platybasic type* (fig. 10.2) the hypothalamic floor is even and the adenohypophysis is attached to the neurohypophysis along its entire dorsal surface (Wingstrand,1966). The infundibular stem is practically absent. The pituitary is placed close to the hypothalamic floor (Holmes and Ball,1974). An extreme example of this type is seen in the gobiidae (including *Lepidogobius*). In the *leptobasic type* (fig. 10.3) the neurohypophysial processes proceed from the top



of a funnel-like depression of the hypothalamic wall, called hypophysial stem (Wingstrand, 1966). The stem enters the adenohypophysis from the dorsal/anterior/posterior aspect. In *Lophius* and *Ipnops* (Wingstrand, 1966) "the pituitary is situated far anteriorly in front of the brain and is connected with the hypothalamus by a long narrow, nerve-like stem". The saccus vasculosus is usually present. It is separated from the hypophysial area by an unmodified brain wall.

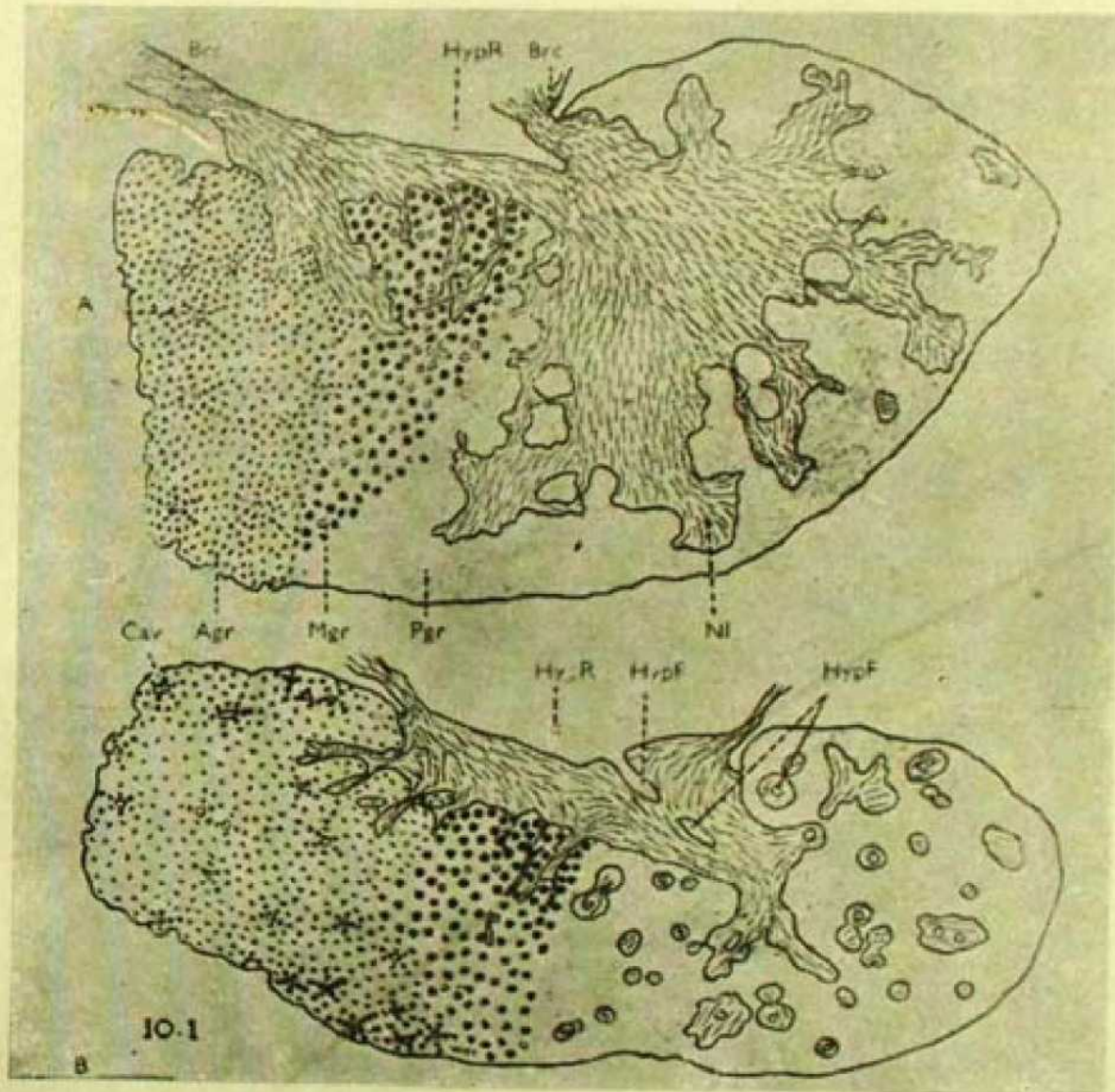


Fig. 10.1. Schematic drawing of the hypophysial structure. (A), *Salmo salar*; (B), *Anguilla anguilla*. (Agr), anterior lobe; (Brc), connexion with the base of the brain; (Cav), cavity in the anterior lobe; (HypR), infundibular recess with diverticulum; (HypF), penetration of the posterior lobe by the diverticulum of the neural lobe; (Mgr), middle lobe; (O), acidophils; (●), basophils; (NI), neural lobe; (Pgr), posterior lobe.—From Vivien (1958). Courtesy of Masson et cie.

Holmes and Ball (1974) said, "In the more primitive teleosts (the isospondylous forms, including clupeoids, salmonids and apodes) the LTH cells are arranged in follicles, as in the eel, and as in the living ganoid fishes which are related to the ancestors of teleosts". In the herring (*Clupea*) a lumen is found in the early anlage of the compact adenohypophysis and an orohypophysial duct is present



until sometime after the metamorphosis of the larva (Wingstrand, 1966). Such a duct is present in the primitive teleost *Elops saurus* (Olsson, 1958) and in young specimens of *Chanos chanos* (Tampi, 1951, 1953). It is also present in *Hilsa ilisha* (Sathyanesan, 1963). The condition is similar to that noted in *Polypterus* and *Calamoichthys*. The follicles present in the proadenohypophysis of the primitive teleosts such as *Salmo*, *Clupea* and *Anguilla* may be regarded as remnants of a

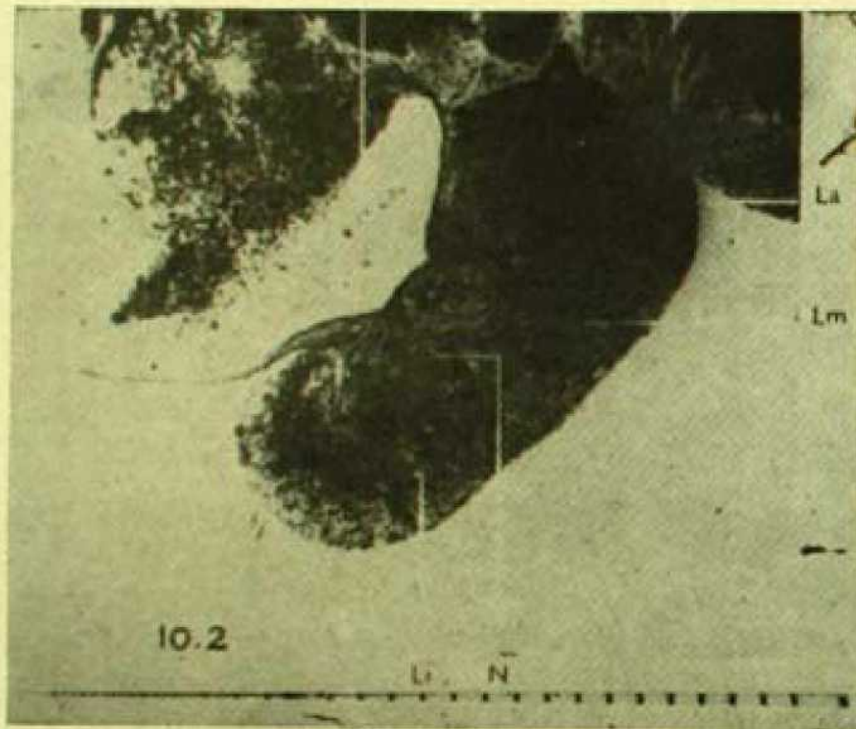


Fig. 10.2. Hypophysis of *Xiphophorus helleri*. (III), infundibulum; (La), anterior lobe; (Lm), middle lobe; (Li), intermediate lobe; (N), ramifications of neural lobe.—From Vivien (1958). Courtesy of Masson et cie.

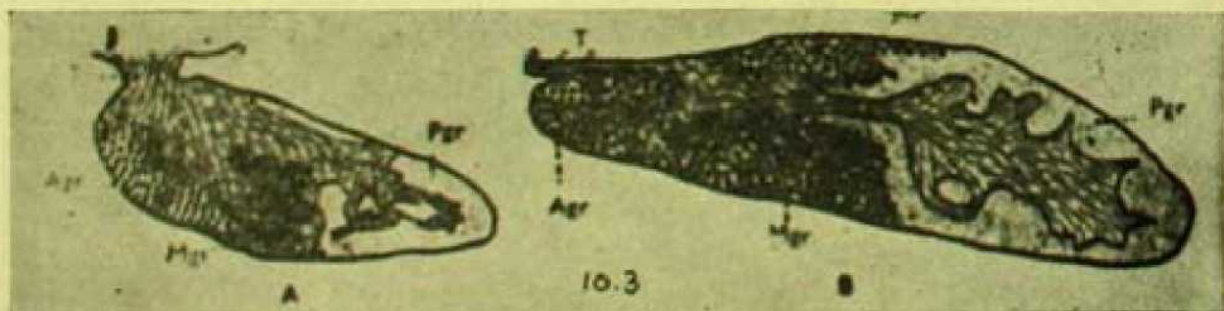


Fig. 10.3. Schematic drawing of hypophysis of *Phoxinus laevis* (A) and *Cobitis* (B). (T), pituitary stalk. (Agr), anterior lobe; (Mgr), middle lobe; (Pgr), posterior lobe.—From Vivien (1958). Courtesy of Masson et cie.

hypophysial cavity. They arise as schizocoelic spaces in the originally compact anlage or by budding from other follicles. However, "the conditions in sturgeons indicate that the follicles may have arisen phylogenetically from glandular tubules, communicating with the hypophysial cavity". A typical pars tuberalis is absent.



Pickford and Atz(1957) reviewed the histology and physiology of the adeno-hypophysis. The mesoadenohypophysis is very much like the tetrapod pars distalis. The cell population includes basophils, eosinophils and chromophobes. Two types of basophils and two types of eosinophils could be differentiated. The proadenohypophysis has fuchsinophilic cells. Metaadenohypophysial cells are chromophobic or basophilic.

"Diepen(1954, 1955, 1962) suggested that the anterior ramifications of the neurohypophysis, which consist of mainly non-neurosecretory fibres, should be regarded as a modified eminentia mediana (infundibulum) since they represent a kind of proximal adeno-neurohypophysial contact" (Wingstrand,1966).

*Blood supply*—Ball and Baker(1969) summarized the observations of different authors regarding the blood supply of the pituitary gland in teleosts. Holmes and Ball(1974) reported the observations upto 1974. Capillaries form a vascular plexus within the neurohypophysis near the adenohypophysial boundary or at the junction of the neurohypophysis and adenohypophysis (Follenius, 1961, 1962; Jasinski, 1961, 1962). The plexus in the neurohypophysis is called the *primary longitudinal plexus or system of Follenius*(1965). Bhargava(1968) studied the intrahypophysial vascularisation and the hypothalamo-hypophysial vascular relationship in the minnow—*phoxinus phoxinus* L. He said, "In the adult conditions the blood vessels in the pituitary stalk are joined to a central longitudinal blood vessel or sinus in the mesoadenohypophysial region of the neurohypophysis. This blood vessel is not well defined at the 4.5cm. stage. In the adults, during the prespawning and spawning periods (February to May-June), this blood vessel is extremely enlarged and this may indicate the functional importance of the blood vessel during this period".

Bhargava(1968) said that the ventral hypothalamic artery is probably the median pituitary artery of Barrington(1960) and just posterior to the optic chiasma, the anterior hypothalamic artery starts. This artery divides into two branches and they are situated by the side of the preoptic recess. They supply the ependymal lining of the ventricle, the preoptic recess and the ventral part of the telencephalon. The ventral hypothalamic artery while coursing backwards gives off a median branch: the posterior hypothalamic artery. The latter divides into two median vessels, one anterior and the other posterior branch. The posterior branch is known as ventral infundibular artery. This artery gives off paired branches to the ependymal lining of the ventral part of the infundibular cavity or recess. The ventral infundibular artery forms a capillary blood plexus with the ring vessels just above the pituitary stalk and then enters into the pituitary gland. The ventral infundibular artery is Barrington's (1960) lateral pituitary artery. The ventral hypothalamic artery then divides into a pair of ring vessels. The ring vessels proceed backwards within the meninges up to the pituitary and enter the nervous tissue of the gland and are connected with the ventral infundibular artery and forms a capillary connection just above the pituitary stalk. In the stalk region branches arise from the ring vessels and they join with the ventral infundibular artery and with one another and enter the pituitary. The vessels in the stalk



region are connected with blood capillaries in the pituitary gland. There is no venous supply of the pituitary except a small branch at the posterior end. The left pituitary vein is larger than the right one. "The neurohypophysis has its independent blood supply though indirectly irrigating the adenohypophysis. On the basis of blood supply, therefore, the neurohypophysis (its distal portion) in the minnow is comparable with the neural lobe of the higher vertebrates." No hypophysial portal system exists in the minnow.

Gomori's CAH positive cells were described by Barrington (1960) just above the pituitary stalk in the minnow. Blood capillaries formed loops and connected the two ring vessels near these cells. The stainable granules were also noted by Barrington in these blood capillaries. Therefore, he thought this part of the hypothalamus to have resemblance to the median eminence of higher vertebrates. Bhargava (1968) did not agree with this finding of Barrington (1960). The median eminence of higher vertebrates is situated in front of the stalk and the infundibular recess but Bhargava noted the Gomori's chrome-alum haematoxylin positive cells to lie posterior to the infundibular recess in the minnow. These cells occupy the same place in the hypothalamus as the nucleus lateralis tuberis of other fishes.

Green (1951) could not find a portal system in the fish as is noted in tetrapods. He said that the blood capillaries or sinuses between the adenohypophysis and neurohypophysis in the fish may take up the same function as the portal system in higher vertebrates and thus the neurohypophysis of the fish can be compared to the median eminence of higher vertebrates.

Hypothalamo-hypophysial vascular relationship was studied by Kerr (1942), Oliverau (1954), Miller (1944), Bretschneider and de Wit (1947) (fig. 10.4), and Pickford and Atz (1957). Bretschneider and de Wit (1947) described the vascularity through the arteria infundibularis superficiales and the arteria infundibularis internae. The centrifugal blood flow from the primary longitudinal plexus in the neurohypophysis is contrary to the method of flow proposed by them. Possibly these vessels correspond to the subhypothalamic cephalic artery of Pickford and Atz (1957).

Follenius and Porte (1962) studied the appearance, ultrastructure and distribution of the neurosecretory material in the pituitary gland of two teleost fishes *Lebistes reticulatus* R and *Perca fluviatilis* L. They said that the vascular organization of the teleost hypophysis differs greatly from that of other vertebrates. The arterial blood courses at first through the longitudinal arterial network in the neurohypophysis (Follenius, 1961). Centrifugal capillaries start from this network and supply the different hypophysial lobes. The centrifugal capillaries are embedded in processes of the nervous tissue which invaginate into adenohypophysis. Neurosecretory fibre bundles are grouped round these capillaries and follow them for some distance. The fibre endings abut against the pericapillary basement membrane or against the limiting basement membrane of the process. Here they have neurosecretory droplets, mitochondria of different shapes and plenty of synaptic vesicles. No perivascular space is present in *Lebistes*. Neurovascular contacts



in all the capillaries of the hypophysial lobes are obtained. Nervous processes containing neurosecretory fibres have been found in the region of the *chromophobic* zone of the proadenohypophysis of *Lebistes*. The nerve terminals are also noted in the mesoadenohypophysis. The neurovascular contact is greatest in the posterior part of the pars nervosa. The blood directly passes into the hypophysial vein and a small quantity may pass through the metaadenohypophysis before reaching the hypophysial vein. Thus in the pro and mesoadenohypophysis the neurosecretory material crosses the hypophysial cells before reaching the superficial venous system. So this neurovascular system plays a role similar to the portal system of tetrapods which is absent in most teleosts (Follenius, 1961).

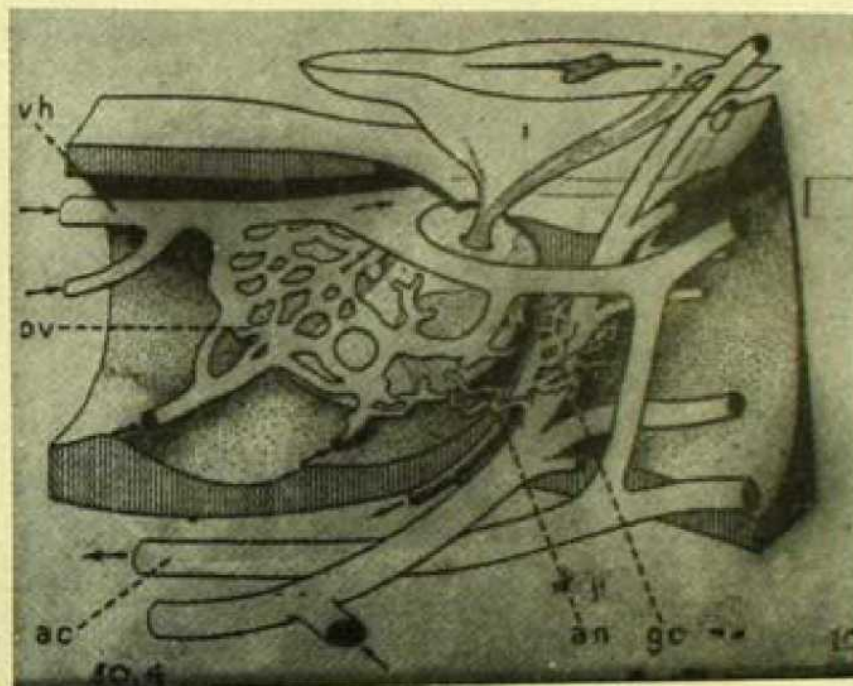


Fig. 10.4. Scheme of hypophysial vascularization of *Rnedeus amarus*. (ac), carotid artery; (an), nourishing artery of the hypophysis; (gc), carotid glomus and vein; (i), infundibulum; (pv), hypophysial venous plexus; (vh), hypophysial vein (Bretschneider and Witt).—From Vivien (1958). Courtesy of Masson et cie.

Follenius and Porte(1962) suggested the following pathway for the active principles of nervous origin controlling adenohypophysial activity.

“(a) Directly through the basen.ent membrane to reach the acidophil cells of the mesoadenohypophysis and the PAS-positive cells of the metaadenohypophysis (Follenius and Porte,1961).

(b) indirectly through a vascular relay in the neurohypophysis of the teleost fishes to reach the other types of cells”.

A typical plexus intermedius is formed which is enclosed by the double basenent membrane in the part of the pars intermedia (Ball and Baker, 1969; Henderson, 1969; Knowles and Vollrath, 1966; and Leatherland, 1970).



Henderson(1969) described the vascular system of the pituitary of the brook trout, *Salvelinus fontinalis*. Two independent vascular beds exist within the gland :

A. Two ventral hypothalamic arteries supply the anterior hypophysis. These arteries divide into a superior ramus and an inferior ramus. The superior ramus supplies the area of the nucleus lateralis tuberis. Arterioles from the inferior ramus form loops in the meninx over the hypothalamus. As the velocity of blood depends on peripheral resistance and as it is increased in the loops, so the flow of blood is retarded in the loops which helps in the neurovascular exchanges in the neurohypophysis. The primary longitudinal plexus is formed by these arterioles and by some branches of the superior ramus. The pars distalis is supplied by branches from this plexus which communicate with the sinusoidal capillaries in the gland. The venous drainage is to the superficial venous plexus.

B. The caudal hypothalamic artery supplies the posterior aspect of the pituitary and the saccus vasculosus. The plexus intermedius is being supplied by branches from the caudal hypothalamic artery. Direct supply to the pars intermedia is very small. The venous outflow is to the superficial venous plexus.

Few arteries from the internal carotids directly supply the adenohypophysis. The dorsal and the posterior aspect of the neurohypophysis may get vascular supply from the longitudinal plexus situated anteriorly and also from the caudal hypophyseal artery.

Holmes and Ball(1974) said, "The anterior primary longitudinal plexus with its ramifications probably has the functions of a hypophyseal portal system, and as in the median eminence of other vertebrates most of the neurosecretory axons associated with this plexus are type B".

Henderson(1969) observed aldehyde fuchsin-positive fibre endings in the region of the plexus intermedius.

Sathyanesan and Haider(1971) described portal system in *Heteropneustes fossilis* and Sathyanesan(1972) described the same in *Clarias batrachus*. However, Sundararaj and Viswanathan(1971) could not find portal system in *Heteropneustes fossilis*.

*Diencephalic neurosecretion and its relationship with adenohypophysis in teleosts*  
(Zambrano, 1972; Holmes and Ball, 1974; Batten and Ball, 1977; and  
Batten *et al.*1979)

Lofgren(1959, 1960) showed that the infundibular cavity of the rat is lined partially by ependymal cells which vary from those covering the other parts of the third ventricle. Activity at the apex of these cells was thought to be showing phagocytosis of the contents of the infundibular recess. Infundibular recess is filled with neurosecretory material and the ependymal cells absorb it. Conduction of the neurosecretory material through these phagocytic cells to the capillaries of the portal system was by means of a protoplasmic filament terminating on



these vessels. Stahl and Leray(1962) said that in mammals, "thus a true ependymo-vascular pathway would permit the transport of tuberal neurosecretory material to the adenohypophysis." These authors studied the brains and hypophysis of *Mugil cephalus*, *Mugil capito*, *Mugil auratus*, *Morone labrax*, *Scorpaena scrofa*, *Scorpaena porcus*, *Diplodus annularis*, *Gadus capellanus*, and *Hippocampus guttulatus*. They could find that the ependymal cells lining the infundibular recess in these fishes are different from the cells lining other parts of the ventricle. Ependymal layer over the preoptic nucleus is formed by apparently inactive flat cells. In the infundibular recess the cells are high and globular. The granules at the apical poles of these cells are azocarmine and PAS-positive. At times there are large globular protrusions which are surmounted by vesicles having fine granules. The basal prolongations of these cells proceed towards the adenohypophysis or towards the tuberal area. Tuberal neurosecretion enters into the infundibular cavity. Basal filaments of the ependymal cells may be in contact with blood vessels. Van de Kamer and Verhagen(1954) and Van de Kamer(1955) described similar type of ependymal cells with apical vesicles in the posterior recess of *Scyliorhinus caniculus*. Van de Kamer thought of the possibility of an absorptive function for these cells.

Knowles(1965) proposed that there are two main classes of neurosecretory system: Type A consisting of fibres releasing peptide substances (peptidergic) and type B (aminergic) fibres. In the teleost fish *Tinca*, a single or double basement membrane separates the fibre terminals from the pituitary cells (Vollrath, 1967). In the eel intervascular or perivascular spaces intervene between the neurosecretory fibre endings and the intrinsic endocrine cells of all the parts of the adenohypophysis. In such cases a few fibres can influence a large number of endocrine cells by the neurosecretory hormones diffusing over a wide area when they are released into the perivascular spaces (Knowles,1971). In *Scyliorhinus* or *Hippocampus* the pituitary cells are directly innervated.

Bern, Zambrano and Nishioka(1971) compared the innervation of the pituitary of two euryhaline teleost fishes, *Gillichthys mirabilis* and *Tilapia mossambica* with special reference to the origin and nature of type B fibres. Type A axons of Knowles(1965) contain typical elementary neurosecretory granules (ENG). They are found directly adjacent to MSH producing cells. Type B axons contain large dense-cored or granulated vesicles(LGV). They make different types of contacts with adenohypophysial cells. "The pituitary of *Gillichthys* is partially embedded in the brain and the neurohypophysis forms a dorsolateral cap, whereas the pituitary gland of *Tilapia* is stalked and lies in a hypophysial fossa.

In both the species, projections of neurohypophysial tissue is surrounded by a thick basement membrane. These projections penetrate into several parts of the adenohypophysis. Axons containing ENG and LGV are adjacent to the neurohypophysial capillaries in *Tilapia*. In *Gillichthys* there are many axons having LGV(90-100nm in diameter) which are associated with capillaries of the neurohypophysial processes. In these cases flattened processes of perivascular cells (Stellate cells in *Tilapia*) always separate the capillaries from these axons. In



this area no direct neurohaemal contact was observed. In the rostral lobe of adenohypophysis of *Gillichthys* the type B axons course through the basement membrane and directly innervate the prolactin cells and ACTH cells. In *Tilapia* thick basement membrane and glia-like cells separate the nerve terminals from the prolactin cells and ACTH cells. Different types of mesoadenohypophysial cells are directly innervated by type B axons in both the species. In *Gillichthys* most of these contacts are of synaptoid nature. Metaadenohypophysial cells (MSH cells) are innervated by type A axons having typical ENG and type B axons. Synaptoid contacts were noted in *Gillichthys*.

LGV in typeB fibres of *Gillichthys* have a strongly positive reaction to zinc iodide-osmium tetroxide (ZIO). The reaction with typical ENG in type A fibres and the secretory granules of adenohypophysial cells is negative with ZIO. After reserpine treatment the dense core of LGV in typeB fibres looks pale after double fixation with aldehydes and osmium. The dense core is totally absent with only osmium tetroxide fixation.

Green to Yellow fluorescence was noted among the cells of the proadenohypophysis of *Gillichthys* with Falck-Hillarp technique. In *Tilapia* fluorescence was noted mostly in the neurohypophysis. Zambrano(1970) noted that the lateral and rostral parts of the nucleus lateralis tuberis are formed by secretory-appearing neurons. These neurons have granulated vesicles of the same size as noted in typeB axons in the pituitary gland and they give a positive reaction with ZIO and E-PTA. These neurons showed a strong green-to-yellow fluorescence with Falck-Hillarp technique. The distribution pattern and situation closely correlated with the distribution of LGV in the nucleus lateralis tuberis (Zambrano, 1970).

Typical retrograde degeneration in the neurons of the rostral and lateral parts of the nucleus lateralis tuberis of *Gillichthys* was noted two days after hypophysectomy. The evidences were the disappearance of the endoplasmic reticulum, swelling of mitochondria, disruption of Golgi membranes and presence of peripheral empty vacuoles. Zambrano(1970) could find almost complete recovery at two weeks after hypophysectomy.

The authors concluded that typeB fibres containing LGV and innervating different adenohypophysial cells in the above mentioned fishes are monoaminergic and they are the storage sites of the active monoamine. In the dense core an active catecholamine is present which is evidenced by positive ZIO reaction and fluorescence reaction. It also contains a carrier protein as evidenced by positive E-PTA reaction and a paler core after reserpine treatment. The authors could find a similarity of typeB fibres regarding the morphology and histochemistry with the adrenergic fibres showing neurohaemal contacts in the outer layer of the median eminence of lower actinopterygians, lungfish and tetrapods (Lagios, 1968; Hayashida and Lagios, 1969; and Kobayashi and Matsui, 1969).

Observations of Zambrano(1970) after hypophysectomy indicate that typeB LGV containing fibres take their origin from the neurosecretory neurons of the



nucleus lateralis tuberis. Urano(1971) confirmed this by demonstrating monoamine oxidase(MAO) activity in the Japanese eel, *Anguilla japonica* and in the medaka, *Oryzias latipes*.

Zambrano, Nishioka and Bern(1972) concluded that the pituitary gland of teleost fishes is directly innervated by two types of neurosecretory fibres. Type A fibres containing elementary neurosecretory granules, are restricted mostly to MSH secreting cells. All glandular cell types are innervated by typeB fibres containing LGV. After incubation with 5-Hydroxydopamine, LGV from typeB fibres show a higher density than controls. Such fibres undergo degeneration after injection of 6-Hydroxydopamine. These results confirm that typeB fibres are monoaminergic. Secretory activity of gonadotrophs, prolactin cells, corticotrophs and MSH cells is regulated by typeB fibres and this has been experimentally proved. The authors said, "The typeB fibres can be considered as the final common pathway linking the nervous and endocrine systems in teleosts."

TypeB fibres originate from cell bodies of the nucleus lateralis tuberis in the teleost *Gillichthys* (Zambrano,1971). The fibres innervate gonadotrophic cells. They (cells and fibres) show increased activity after castration. Androgen replacement therapy abolished this effect. Regulation of gonadotrophic activity was also suggested by Knowles and Vollrath(1966) in the eel and by Peter(1970) in the goldfish.

Prolactin cells of teleosts are under inhibitory hypothalamic control (Sundararaj and Nayar,1969; Sage,1970; Zambrano,1971). Zambrano(1972) found this control to be mediated by typeB fibres. Zambrano *et al.*(1972) destroyed typeB fibres of the teleost *Gillichthys* by 6-HODA treatment. The prolactin cells became hypertrophied with evidences of increased synthesis. The nuclear membrane is highly infolded and there were plenty of mitochondria. The endoplasmic reticulum hypertrophied and there were active Golgi complexes. Mitotic figures were also noted. The pituitary gland (rostral lobe) of Japanese masu salmon, *Oncorhynchus masou* in the parr and smolt stages was also studied because the two forms show different adaptive properties while put into a marine environment (Utida and Hirano,1971). Inverse relationship between the neurosecretory activity of typeB fibres and the activity of prolactin cells was noted. In the parr there were ultrastructural evidences of increased synthesis and release of the secretory product of prolactin cells. In the smolt stage inverse picture is noticed. TypeB fibres are filled with plenty of LGV and the prolactin cells are less active. The finding of very active prolactin cells in the parr and much less active cells in the smolt indicates that the parr is not adapted to life in sea water but the smolt is already prepared for subsequent marine life (Utida and Hirano,1971). Ball(1969) showed that prolactin cells become active in freshwater-adapted euryhaline fishes.

Zambrano *et al.*(1972) also studied the changes in ACTH and MSH secreting cells. ACTH cells have dense granules which are smaller than those of prolactin cells. MSH secreting cells are elongated and there are plenty of pale granules



and dense granules are few. The ER cisternae and Golgi apparatus are poorly developed. Increased activity in both cell types was noted after 6-HODA treatment. In the rostral lobe type B fibres showed different degrees of degeneration. The observations indicate that an adrenergic system inhibits MSH secretory activity. Increased activity of corticotrophs after 6-HODA treatment is associated with increased plasma level of cortisol in *Gillichthys*. This rise may also be due to high titres of MSH. In mammals ACTH secretion is tonically inhibited by a central adrenergic neural system (Van Loon *et al.*, 1971).

Some dispute still exists regarding the fibres which innervate the teleost adenohypophysis. By autoradiographic electron microscopy Follenius (1970) studied the uptake of  $^3\text{H}$ -noradrenaline in the nerve terminals in the stickleback (*Gasterosteus aculeatus*). Noradrenaline uptake by the nerve endings in the cells of the rostral pars distalis including the ACTH cells could not be demonstrated. Labeled noradrenaline was present only in the nerves which richly innervated the cells of the pars intermedia. Follenius therefore, concluded that the innervation of the rostral pars distalis is not adrenergic. Similarly monoaminergic denervation had no effect on the ultrastructure of the ACTH cells or plasma cortisol level in *Tilapia mossambica* (Zambrano *et al.*, 1973/74). No ultrastructural change was noted in ACTH cells of *Gillichthys* after detopic transplantation of the adenohypophysis (Nagahama *et al.*, 1974).

Lederis (1964) showed that the rainbow trout elaborated isotocin (ichthyotocin) and AVT. There were two populations of granules: one of about 140nm and the other of about 180nm. Rodriguez (1971) said that isotocin may be stored in the 140nm granules because the 180nm granules are likely to store AVT.

Vollrath (1972) studied the median eminence of the eel with the help of electron microscope. It consists of non-myelinated nerve fibres with different types of vesicles and granules. Perikarya with electron-dense granules of 1,200-1,800Å in diameter are found rarely. Ependymal cells line the infundibular recess and project into the median eminence and glial cells resembling protoplasmic astrocytes are also found. Direct contact between the nerve fibres of the median eminence and the intrinsic endocrine cells are usually not found but they are separated by a space 3,000-6,000Å in width lined by two basement membranes. Fibroblasts, collagen, reticular fibres and blood capillaries are contained in this space. Processes of glial cells project on to the basement membrane. Nerve fibres also abut against the basement membrane. The mesoadenohypophysis contains gonadotrophic and somatotrophic cells. There are three types of fibres.

Group I fibres: comprise electron-dense vesicles of Ca.800Å in diameter and electron-lucent vesicles of Ca.500Å.

Group II fibres: comprise only electron-lucent vesicles of about 500Å in diameter.

Group III fibres: comprise electron-lucent vesicles of 250-350Å. In all the three groups a few electron-lucent vesicles of 500-800Å are found.



The pro-adenohypophysis contains adrenocorticotrophic, thyrotrophic and prolactin cells. Two types of fibres could be found.

Type I fibre : comprises electron-lucent vesicles of Ca.500Å in diameter.

Type II fibre : contains electron-dense granules of 600-800Å in diameter and electron-lucent vesicles of Ca.500Å in diameter.

Vollrath(1972) studied the changes after experimental manipulations.

*Metopirone* (200mg/kg) for 1-3 days resulted in increased production of ACTH due to inhibition of adrenal function. The nerve fibres close to such ACTH cells contained either increased or decreased number of electron-dense granules. Secretory granules of 1,400Å in diameter were not found.

*Hydrocortisone* (100mg/kg) administration (after one day) did not show any change in the nerve fibres of the ACTH region. ACTH cells showed increased lysosomes. Two to three days after, more dense-core and electron-lucent vesicles could be found. Metopirone or hydrocortisone did not affect the nerve fibres of the meso-adenohypophysis.

*Estradiol-benzoate* at a single dose of 4mg/kg administration showed some changes in the projections extending into the gonadotrophic region of the pituitary gland after three days. Moderate increase in the number of electron-dense granules of 800Å in diameter was found. Some nerve fibres contain a large number of electron-dense granules, 1,200-1,800Å in diameter with varied shapes.

*Reserpine* (2-4mg/kg for 1-3 days) administration resulted in complete disappearance of electron-dense granules of ca.800Å in diameter from some areas of the median eminence and in others there was only slight decrease.

Vollrath(1972) concludes that a close correlation exists between the nerve fibres of the median eminence and the intrinsic endocrine cells. Corticotrophin releasing factor has been studied by Roy(1964) and Sage and Purrott(1969) in the teleost fish. In the teleost fish thyrotrophin inhibitory factor has been described by Peter(1970). Vollrath(1972) thinks that electron-lucent vesicles of the size range of synaptic vesicles, dense-core vesicles of 600-800Å in diameter and also of 1800Å in diameter may be a probable source of releasing factors.

The structure of nucleus preopticus and nucleus lateralis tuberis of *Salmo salar* and *Salmo gairdneri* was studied by Terlou and Ekengren(1979) and their relationship to the hypophysis was established.

Dubois(1976) reviewed the immunocytochemical evidence of LH-RH in hypothalamus and median eminence. Crim *et al.*(1978) obtained strong evidences for the presence of LHRH-like activity in central nervous tissues of some teleosts. Dubois *et al.*(1979) used immunocytochemical method to study the comparative distribution of somatostatin, LHRH, neurophysin and  $\alpha$ -endorphin in the rainbow trout. By immunofluorescence method evidence of a somatostatin (SRIF)-like antigen was found in the brain and digestive tract. In the diencephalon, the peri-



ventricular SRIF immunoreactive hypendymocytes are situated dorsal to the nucleus preopticus. ir-SRIF perikarya are concentrated in the nucleus preopticus periventricularis anterior to the nucleus preopticus. They are scattered in small cells in the nucleus lateralis tuberis pars anterior and in a few cells situated in an unnamed nucleus in the dorsomedial hypothalamus. In the pituitary, ir-SRIF is situated in the neurohypophysial tissue in the proximal pars distalis. SRIF + cells have been noted in the endocrine pancreas and the gastric mucosa. LHRH has the same distribution pattern in the pituitary as SRIF. Neurophysin immunoreactivity was noted only in the neurophysial tissue of the neurointermediate lobe. Few cells reacted with anti- $\alpha$ -endorphin in the nucleus lateralis tuberis in the pituitary stalk region. All pars intermedia cells in the neurointermediate lobe reacted with anti- $\alpha$ -endorphin. Follenius and Dubois(1980) described anti- $\alpha$ -endorphin +, and anti-Met-enkephalin + pathways in the brain and pituitary of carp (from Ball and Batten, 1980).

Hunter and Baker (1979) noted the distribution of opiate activity in the trout pituitary gland. Naloxone-reversible, opiate activity was found in the pars distalis and neurointermediate lobe having similar total activity in each and the concentration was approximately equal to that found in the guinea pig neurointermediate lobe. The relationship between opiate concentration and cellular activity in the pars distalis and neurointermediate lobe of the eel pituitary was studied by Carter and Baker (1980).

#### *Morphological cell types of the pituitary of the eel*

These have been recently described by Olivereau and Herlant(1954, 1960), Olivereau(1960, 1960/61, 1961, 1963, 1965, 1966), Olivereau and Fontaine(1966), Knowles and Vollrath(1966), Vollrath(1966), Holmes and Ball(1974), Fontaine and Olivereau(1975), and in subsequent publications of Olivereau and Ball. The rostral pars distalis (proadenohypophysis) has a follicular structure (fig. 10.8). The small vesicles are surrounded by rosettes of fusiform cells. The cells are acidophils with fine erythrosine and orange G-positive granules. The acidophils are also rich in sulphhydryl groups. Some of the follicle cells and angular cells situated in the interfollicular spaces have fine basophilic granules which are alinine blue, PAS, AB, AF and aldehyde thionine-positive. Neurohypophysial protrusions into the dorsocaudal part of the rostral pars distalis are lined by cuboidal cells. The fine granules of these cells are fuchsinophilic, erythrosinophilic and acid alizarine blue positive. This acidophil has lead haematoxylin positive granules.

In the proximal pars distalis (mesoadenohypophysis) groups of basophils are scattered among cords of acidophils. These acidophils are rounded having medium sized granules which are erythrosinophilic and orangeophilic. They are rich in sulphhydryl groups. The rounded or elongated basophils have aniline blue, PAS, AB and AF-positive granules.

Pars intermedia (metadenohypophysis) contains two cell types. The frequent cell type is an elongated cell which is perpendicular to the branch of the



neurohypophysis. The granules are orangeG, aniline blue, acid alizarine blue and lead haematoxylin-positive. They are PAS, AB and AF-negative. The other type of cells is situated in the centre of the cell cords. These cells are small, angular and the granules are PAS-positive and AB, AF and lead haematoxylin-negative.

### *Morphological cell types in other groups*

Cell types are similar but they have minor differences.

<i>Salmo salar</i>	Fontaine and Olivereau(1949) Olivereau(1954, 1964) Gabe(1958)
<i>S. gairdnerii</i>	Gabe(1958) Robertson and Wexler(1962) Olivereau(1964) Olivereau <i>et al.</i> (1964) Olivereau and La Roche(1965)
<i>S. fario</i>	Baker(1963)
<i>Oncorhynchus keta</i>	Olivereau and Ridgway(1962) Olivereau(1964) Barannikova(1964)
<i>O. nerka</i>	Robertson and Wexler(1962) Olivereau and Ridgway(1962)
<i>O. tshawitscha</i>	Robertson and Wexler(1962) Olivereau(1964) Olivereau and La Roche(1965)
<i>Clupea harengus</i>	Buchmann(1940)
<i>Murena helena</i>	Bugnon(1960)
<i>Conger conger</i>	Knowles and Vollrath(1966)

The minor differences are that the lead haematoxylin positive cells of the rostral pars distalis have less affinity for erythrosine. The other difference is that



the lead haematoxylin negative cells of the pars intermedia may be chromophobic. These cells are rich in acid phosphatase in the trout (*Legait et al.*, 1964).

*The adenohypophysis of more advanced teleosts.*

It is ovoid in shape and the three parts are situated one over the other and the branchings of the neurohypophysis project deeply into the adenohypophysis and these projections are shorter in primitive teleosts.

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<i>Poecilia reticulata</i>	Sokol(1953, 1955, 1961) Geske(1956) Vervoort(1957) Folpenius(1959)
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<i>P. formosa</i> , and	Oliveriau and Ball(1964)
<i>P. latipinna</i>	Ball and Baker(1969)

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<i>Xiphophorus maculatus</i>	Oztan(1961) Schreibman(1964)
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<i>Fundulus heteroclitus</i>	Sokol(1961) Emmart <i>et al.</i> (1966)
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No follicular arrangement is seen in the rostral pars distalis, rather the cells form strands or clusters. The most prominent cell type is rounded or angular and the granules are strongly erythrosinophilic. The neurohypophysial projections are lined by tall cells with faint staining of the granules by acid alizarine blue and lead haematoxylin. Three cell types are found in the proximal pars distalis. A single acidophil cell type and two basophil cell types are also present. The acidophils are situated dorsally between the projections of neurohypophysis. They are rounded in appearance. The granules are large and deeply orangeophilic. Groups of spherical basophils are scattered among these cells. The fine granules are aniline blue, PAS, AB and AF-positive. The second group of basophils are situated in the periphery of the ventral part of the proximal pars distalis. They are round or irregularly shaped. The staining reaction of the granules is same as in the other type of basophil. The ventral basophils in *Xiphophorus* have globules which are orangeophilic and carminophilic over and above the basophilic granules (Oztan, 1951; Schreibman, 1964).

The pars intermedia has two cell types. The shape of the prominent cell type is rounded or irregular. There are light erythrosinophilic granules or the



cells are agranular. The other type of cell is small and polygonal. These small groups of cells line the projections of neurohypophysis and the granules are PAS and lead haematoxylin-positive.

Roy(1962) studied the pituitary of *Labeo rohita*, *Cirrhina mrigala* and *Katla katla*. Acidophilic, basophilic and chromophobic cells are present in the proadenohypophysis. Eosinophilic cells are plenty in number. The basophilic cells are very few. No marked change was noted in this lobe during the sexual cycle. The mesoadenohypophysis contains three cell types. Acidophils and basophils outnumber the chromophobic cells which are nongranular. Some of the basophilic cells contain vacuoles and specially in those fishes subjected to stress. Seasonal changes are marked in this zone of the pituitary. The basophils also contain acidophilic globules. These globular basophils increase in size and number and also the globules increase in size during the advent of the season with increased sexual activity. The acidophils become prominent also. Thyrotrophic and gonadotrophic basophils could be differentiated. The cells of the metaadenohypophysis stained pale pink with PAS. There were two varieties of cells: granular and agranular.

Holmes and Ball(1974) discussed the histophysiology of the pars distalis.

Doerr-Schott(1976) immunohistochemically detected pituitary hormones in cold-blooded vertebrates (fish, amphibians and reptiles) by light and electron microscopy. The antisera were raised to purified mammalian pituitary hormones. Schreibman *et al.*(1973) discussed functional morphology of the teleost pituitary gland and considered the eta cells as the source of prolactin hormone in teleost. Schreibman and Holtzman(1975) reported the histophysiology of the prolactin cell in nonmammalian vertebrates.

#### LTH cells (fig. 10.5)

Emmart *et al.*(1966) were the first to localize prolactin within the pituitary of a cyprinodont fish, *Fundulus heteroclitus* (Linnaeus), by specific fluorescent anti-ovine prolactin globulin. The antiserum was found to be specifically bound by the eta cells of the proadenohypophysis (rostral pars distalis).

The cells secrete fish prolactin or paralactin and are engaged in osmoregulation in many teleosts (Ball,1969; Ensor and Ball,1972). They are situated in the rostral pars distalis and the staining reactions have been already mentioned. Holmes and Ball(1974) stated "Fluorescent antibody to ovine LTH locates specifically on the granules of the lactotrophs in various teleosts (*Fundulus*, *Oncorhynchus*, *Cichlasoma*, *Carassius*, *Leuciscus*, *Anguilla*, *Salmo*, *Clupea*) (Ball and Baker,1969; McKeown and van Overbeeke,1971; Mattheij and Sprangers,1969; Emmart,1969; Aler,1970,1971), confirming that the granules themselves do contain LTH".

Ingleton and Stribley(1977) identified cells of origin of eel pituitary hormones separated by polyacrylamide gel electrophoresis by immunofluorescent method.



The antiserum to eel prolactin stained the eta cells in *Anguilla anguilla*. Rawdon (1979) observed immunostaining of eta cells in the rostral pars distalis and PAS+cells in the pars intermedia of a teleost (*Sarotherodon mossambicus*) by antisera to mammalian (human, ovine and bovine) prolactins. Eta cells are the source of prolactin-like hormone. They have antigenic determinants in common with mammalian prolactins. Two of the antisera stained PAS+cells in the

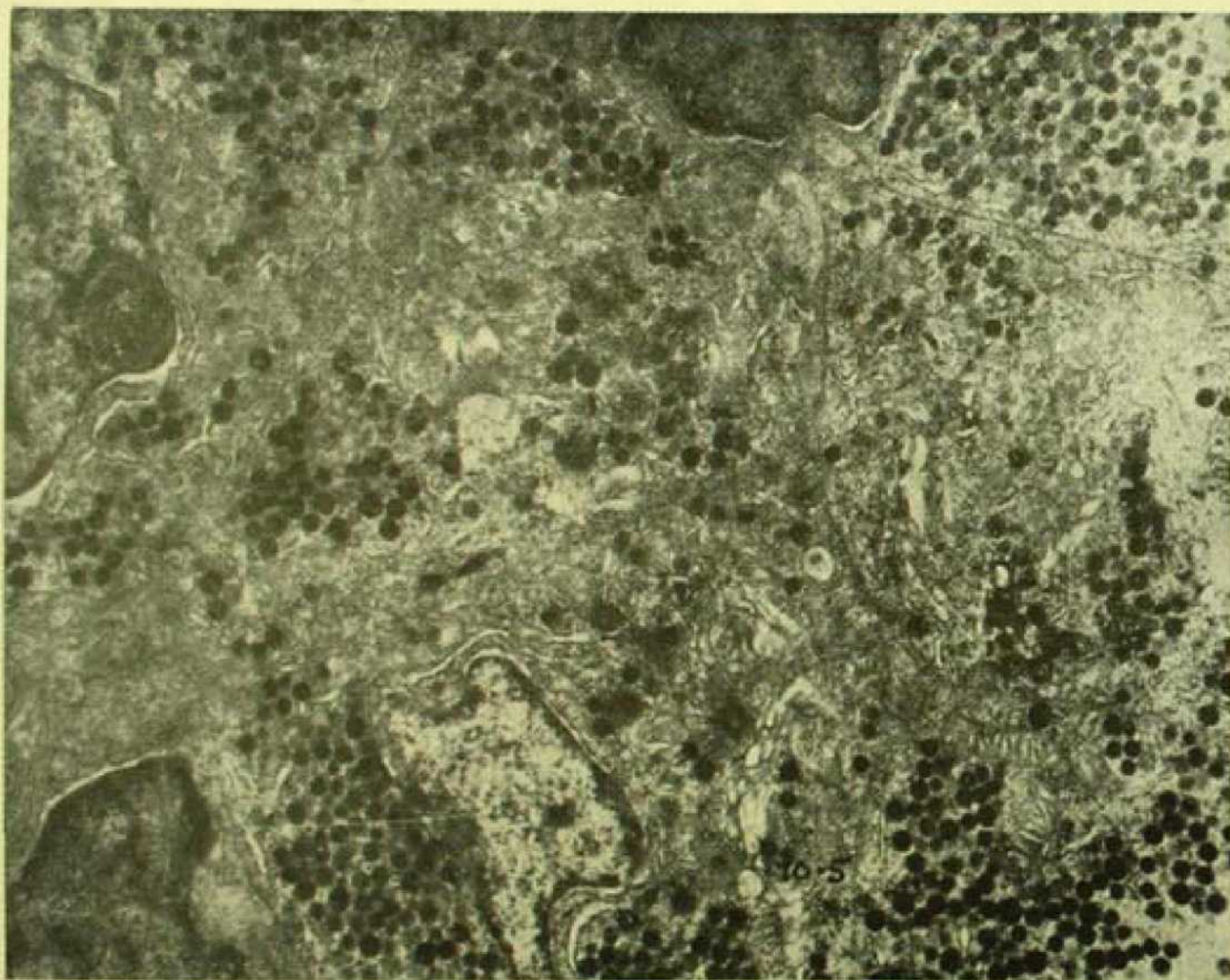


Fig. 10.5. Prolactin cells in the rostral pars distalis of the teleost *Chasmichthys dolichognathus*. Courtesy of Professor Kobayashi and Dr. Tsuneki (1977).

pars intermedia in addition to eta (prolactin) cells. By absorption of the antisera with the appropriate prolactin antigens, staining of both the cell types could be eliminated. The secretory product of PAS+cells either shares antigenic determinants with mammalian prolactins or the antibody which stains these cells is directed against another peptide present as a contaminant in the antigen. The author suggests that further experiments are required to solve this problem.

Ultrastructural studies were undertaken by Dubourg *et al.* (1980) on prolactin cells of *Gambusia* collected in fresh water, in hypersaline medium (45g NaCl/liter), and acclimated to deionized water. In hypersaline medium, reduced activity



(synthesis and release) was noted in prolactin cells. In deionized water, prolactin cells showed considerable stimulation (hypertrophy and hyperplasia). The releasing activity was higher than the synthesizing capacity. No function could be attributed to the agranular cells.

Daily rhythms of liver cAMP, total liver lipids, prolactin-like hormone and growth hormone cell activities in *Sarotherodon mossambicus* acclimated to different photoperiod regimes were studied by Carrillo *et al.* (1980). A significant correspondance could be observed between the prolactin cell cycle and the total liver lipid levels cycle, and between the growth hormone cell cycle and the liver cAMP level cycle.

Farmer *et al.* (1977) found evidences to suggest that "*Tilapia* PRL has features common to both mammalian PRLs and GHs as well as to *Tilapia* GH, lending support to the hypothesis that PRL and GH originated from a common ancestral molecule."

Wigham and Ball (1977) concluded that when hypothalamic connections are lost (ocular pituitary transplants), prolactin cells of *Poecilia latipinna* *in vivo* can be activated by reduced environmental salinity. The time-course of this activation is same as found in intact fish.

Wendelaar Bonga (1978) concluded, "ionic calcium concentration, and not osmolarity or sodium content, is the main environmental factor in the control of prolactin secretion in sticklebacks."

Wendelaar Bonga (1980) studied the effect of synthetic salmon calcitonin and low ambient calcium on plasma calcium, ultimobranchial cells, Stannius bodies, and prolactin cells in the teleost *Gasterosteus aculeatus*. Stannius type 1 cells likely produce a hypocalcemic hormone. Prolactin has a hypercalcemic action.

Factors affecting *in vitro* activity of prolactin cells in the euryhaline teleost *Sarotherodon mossambicus* (*Tilapia mossambica*) were evaluated by Wigham, Nishioka and Bern (1977). Prolactin cells in the rostral pars distalis are directly affected by osmotic pressure because prolactin release from the control tissues was always greater in hyposmotic than in hyperosmotic medium. In hyposmotic medium release of prolactin was inhibited by dopamine but the synthesis was not affected. There was no effect on prolactin secretion by octopamine in hyposmotic medium and by GABA in hyperosmotic medium. Prolactin release was inhibited by cortisol in hyposmotic medium and to a lesser extent in the hyperosmotic medium. Specific prolactin activity was found to be increased only in the hyperosmotic medium. In the same medium no change in prolactin release could be observed by estradiol-17 $\beta$  but there was an increase in synthesis of the hormone. In hyposmotic medium TRH inhibited prolactin release but synthesis was unaffected. In hyperosmotic medium TRH did not affect prolactin secretion. TRH at 100pg/ml was also ineffective in hyperosmotic medium. In hyposmotic medium prolactin synthesis and release was inhibited by somatostatin. Inhibition



of release only was noted in hyperosmotic medium by somatostatin. These experimental observations of the authors indicated a complexity in the regulation of the prolactin cells.

Idler *et al.* (1978) isolated prolactin from salmon pituitary. Prolactin not adsorbed on DEAE Bio-Gel A (DEAE<sub>1</sub>) had a molecular weight of 24,300 by gel filtration and 20,500 by SDS gel electrophoresis. Plasma sodium levels in hypophysectomized *Poecilia latipinna* were consistently maintained by the protein at very low dosage. Antibodies against this prolactin had specific localization in the prolactin cells of rainbow trout pituitary. A protein fraction which was more strongly adsorbed on DEAE Bio-Gel A (DEAE<sub>2</sub>) also had some prolactin activity in the *Poecilia* assay. The fraction had three proteins with molecular weights of 23,000, 46,000, and 66,000 after DEAE chromatography. "Higher molecular weight proteins are aggregates formed during isolation but the 23,000 molecular weight protein may be a modified form of the unadsorbed prolactin." The growth hormone fraction (DEAE<sub>2</sub>) had no prolactin activity. In the *Poecilia* assay DEAE fractions 3 and 5 were also inactive.

In the sea-horse, *Hippocampus* parental role of fish LTH has been observed. There is a ventral brood-pouch in the male sea-horse for incubating the eggs. It is maintained partly by LTH. The lactotrophs of the male fish show an annual cycle having correlation with the development and function of the brood-pouch. These cells are active during the first half of the incubation period (Boisseau, 1967). Lactotrophs are found to be inactivated by ACTH treatment. Corticotrophs show an annual cycle of activity. They are most active during the brooding of the eggs in the pouch.

Regarding the control of LTH, Holmes and Ball (1974) stated "The study of LTH-dependent characters in *Anguilla* has suggested that the ectopic gland in fact releases less LTH than normal, and reserpine seemed to induce some inhibition of LTH release. There must certainly be differences among teleosts in the details of control of the pituitary, and at present it is only possible to say that LTH secretion seems to have a large degree of autonomy, but that there may be a hypothalamic LTH-inhibiting factor, with hints of an LTH-stimulating factor, the balance between the two being probably different in different species (Ball *et al.*, 1972)".

In *Xiphophorus* hypothalamic PIF controls prolactin release (pituitary culture) (Sage, 1966, 1968). Ball *et al.* (1972) found the prolactin cells to be under inhibitory control (autotransplantation studies). Hall and Chadwick (1979) reported the control of prolactin and growth hormone secretion in the eel *Anguilla anguilla*. The hypothalamus has prolactin stimulating activity *in vitro*. Horseman and Meier (1979) studied circadian-dependent prolactin effects: hepatic RNA metabolism and prostaglandin mediation. The authors conclude that the late prolactin influence is probably mediated by prostaglandin whereas the early prolactin influence depends upon other mechanisms.



Olcese *et al.* (1979) concluded, "the enzyme MAO may be a significant component of the monoaminergic system of the goldfish, serving to modulate the serotonergic inputs to centres involved in the control of pituitary prolactin secretion". Olcese and de Vlaming (1979) noted *in vitro* estradiol-17 $\beta$  actions on hypothalamic MAO activity in the goldfish (*Carassius auratus*). Low levels of estrogen increased MAO activity and high levels depressed the enzyme activity. This biphasic response may be the result of change in endogenous estrogen levels.

Hypothalamic control of prolactin and growth hormone secretion in different vertebrate species has been studied by Hall and Chadwick (1979) using different vertebrate pituitaries (mammals, birds, reptiles, amphibians) incubated *in vitro* with various hypothalamic extracts (HE). Rat (*Rattus norvegicus*), chicken (*Gallus domesticus*), terrapin (*Chrysemys picta*), and toad (*Xenopus laevis*) pituitaries were incubated with homologous HE. Prolactin release was inhibited by rat HE. In other species HE stimulated prolactin release. HE stimulated GH release in all four species. The release of the hormones was dose-dependent. Incubations of chicken pituitaries were done with chicken HE and rat HE. The rat HE inhibited the chicken HE-stimulated release of prolactin, as measured by radioimmunoassay.

Hypothalamic prolactin releasing/inhibiting factors and GH releasing/inhibiting factors were investigated by using heterologous incubations. HE from the eel (*Anguilla anguilla*), the cod (*Gadus gadus*) and the flounder, (*Pleuronectes flesus*) and also from other species mentioned above were added to the incubation media with chicken pituitaries. Autonomous chicken prolactin release was marginally inhibited by cod and flounder HE. HE from these species dose-responsively inhibited chicken HE-stimulated prolactin release. Chicken HE-stimulated GH release was also inhibited by cod HE. Chicken prolactin release was stimulated by HE from the eel, the terrapin and the toad. "Hormone release from terrapin and toad pituitaries incubated with heterologous HE was consistent with hypothalamic control via releasing factors in these species".

Parachlorophenylalanine (pCPA) is an inhibitor of tryptophan hydroxylase depleting brain serotonin in higher vertebrates. Oliverreau (1978) noted that plasma electrolyte values were not modified after 4 or 6 injections (200 mg/kg/day) or 10 injections (100 and 140 mg/kg/day) in the eel. After six and ten injections prolactin (PRL) cells appeared less active and their nuclear areas were reduced. 5-hydroxytryptophan injections stimulate PRL cells. A serotonergic system may take part in the regulation of PRL cell activity. "Brain serotonin depletion probably decreases granule release in PRL cells, a result comparable to the lowering action of pCPA on the plasma PRL level in some mammals."

Oliverreau (1978) found prolactin synthesis and release to increase with pimozide. Hypothalamic inhibitory control on PRL secretion mediated through dopaminergic fibres exists in the eel. Other factors may also be involved in this regulation in addition to the effect of salinity.



Olivereau(1977) found that the kidney plays a minor role in osmotic adjustment in seawater(SW) when PRL secretion is reduced (intact eels) or suppressed (hypophysectomized); PRL treatment reverses effects of SW adaptation. The high blood sodium level may be due to inhibition of gill sodium extrusion. Renal participation also occurs in eel through reduced water permeability as in *Platichthys*.

Olivereau and Olivereau(1978) observed the effect of ovine prolactin (PRL) treatment in intact eels in sea water (SW). Hypercalcemia occurred with simultaneous modifications in the Corpuscles of Stannius(CSt). The corpuscles had two categories of cells. Type 1 is the predominant cell type, oval in shape having large granules. Hypertrophy of the nucleus and nucleolus and mitotic activity could be noted. PRL greatly stimulates this cell type. "It may elaborate a hypocalcemic factor (hypocalcin) which would compensate for the PRL-induced hypercalcemia. A similar effect, although slightly less intense, is detected in hypophysectomized-PRL treated cells in SW". The type 2 cell is more elongated and smaller in size. The nucleus is oval and the cell contains fine granules. "Scarcely less active in SW, it is significantly stimulated by PRL despite an increased blood sodium and sodium and potassium level. This experiment does not help to clarify its function."

Nicoll(1974) discussed the physiological actions of prolactin in different vertebrates and expressed them in different tables. The actions of prolactin related to reproduction in teleosts are skin mucous secretion (e.g., discus milk), reduction of toxic effects of estrogen, growth and secretion of seminal vesicles, parental behaviour (nest building, fin fanning, buccal incubation of eggs), maintenance of brood pouch in male seahorse, and gonadotropic in nature. Actions of prolactin on specific target cells or tissues in teleosts are proliferation of melanocytes, growth of seminal vesicles, and renal glomerular growth, tubule stimulation and proliferation. Actions involving water and electrolyte balance are survival of hypophysectomized euryhaline freshwater species, restoration of water turnover in hypophysectomized *Fundulus kansae*, restoration of plasma  $\text{Na}^+$  and  $\text{Ca}^{2+}$  in hypophysectomized eels when given with cortisol, skin, buccal, and gill mucous secretion, reduced gill  $\text{Na}^+$  efflux (reduced permeability), reduced gill permeability to water, inhibition of gill  $\text{Na}^+-\text{K}^+\text{ATPase}$ , renotropic (increased glomerular size), increased urinary elimination and decreased salt excretion, stimulation of renal  $\text{Na}^+-\text{K}^+\text{ATPase}$ , decreased water absorption and increased  $\text{Na}^+$  absorption in flounder bladder, and decreased salt and water absorption from eel gut. Nicoll(1974) enumerated the actions of prolactin involving synergism with steroid hormones or on organs also influenced by steroids. In teleosts they are:  $\text{Na}^+$  retention by gills (corticosteroids),  $\text{Na}^+$  retention by kidney (corticosteroids), salt and water movement in gut (corticosteroids), synergism with androgens on catfish seminal vesicles, dispersal of yellow pigment in xanthophores (corticosteroids), and maintenance of brood pouch in male seahorse (corticosteroids).



### ACTH cells

These cells are situated in the rostral pars distalis between the LHT cells and the neurohypophysis. They are very near the projections of the neurohypophysis. Because of their very faint staining, they look like chromophobes. In the eel Olivereau described corticotrophs having coarse and dense granulations they stain heavily with lead haematoxylin and Alizarine blue but more faintly with erythrosin. Olivereau(1970) noted the normal ACTH cells in the carp (*Cyprinus carpio*) but the corticotrophs were atrophic and chromophobic after eight months of fasting. The interrenals became relatively inactive.

Malo-Michele(1979) studied the cytological reactions of the pituitary-adrenocortical axis in *Boops salpa L* (marine teleost) after diminution of salinity, injection of metopirone, reserpine, and neurogenic stress (noise). Similar responses were frequently obtained in the corticotrophic cells of the pars intermedia. The problem of the control and function of pars distalis ACTH and pars intermedia ACTH was also discussed.

Roy(1969) studied the brain mechanisms responsible for ACTH release in the fish (*Ophiocephalus punctatus*). Changes in the ACTH cells have been noted in different experimental conditions. These cells occur in the rostral pars distalis. They cannot be stained with PAS and aldehyde fuchsin but they contain fine erythrosinophilic granules and thus they sometimes escape attention and are taken to be of chromophobic series. The cells, sometimes oval or columnar in type, are abutted against the neurohypophysis. These epsilon cells of Olivereau(1964) manifest either normal or stimulated picture. The atrophic picture was found in near-total forebrain lesion. During stimulation experiment of the brain or stress which were manifested with rise in plasma 17-OHCS content, the layer of epsilon cells increased in thickness with nuclear and nucleolar prominence. The nuclear membrane was thick. Degranulation response and vacuolation of the cells were noted. At times only the nucleus remained and the cytoplasm was completely replaced by vacuole. Mitotic figures were scanty. In near-total forebrain ablation, these ACTH cells showed atrophic or involutionary picture. The thickness of the epsilon cells diminished without any prominence of the nucleus and nucleolus. The nuclear membrane was also thin. The standard of erythrosinophilic granules was in between the normal and hypersecreted cells or sometimes the cytoplasm became compact and homogeneous. No mitotic figures were noted. Olivereau(1964) and Ball and Olivereau(1966) concluded that the epsilon cells of the pars distalis are responsible for ACTH secretion.

Ball and Baker(1969) said that surgical removal of interrenals in *Anguilla* (Olivereau) and other different treatments prove that ACTH is discharged from this cell type. McKeown and van Overbeeke(1971) showed that fluorescent labeled antibodies to porcine ACTH and synthetic  $\beta$ 1-24 ACTH locate specifically in the epsilon cells of *Oncorhynchus nerka*.



*Pituitary stalk section and experiments with autografting of the pituitary* (Roy, 1964)

The pituitary stalk region of *O. punctatus* was exposed and the stalk was divided by a fine needle. After severance of the stalk the pituitary was left in place and the wound was closed. In another set of experiment the pituitary was grafted into the anterior chamber of the eye after hypophysectomy (autogenous graft). After pituitary stalk section the vascular and nervous connexions with the hypothalamus was disrupted and there was atrophy of the neural lobe component. The anterior lobe was subsequently well vascularized. The gonads in male and female fishes did not show any deviation from normal in whichever part of the year this operation was performed. In fishes with successful grafting of the pituitary in the anterior chamber of the eye, atrophy of the gonads was seen. During the period of observation (upto six weeks) no marked atrophy of the anterior interrenal cells was noted. Thus it seems that the connexion with hypothalamus is important for the integration of the pituitary-gonad-axis and some chemical substance is definitely required for the purpose—it may be from the tuberal nuclei. The autografted anterior pituitary can maintain the anterior interrenal cells.

Ectopic pars distalis of the eel has greater autonomous corticotrophic activity than that of the *Poecilia* (Olivereau, 1971).

Olivereau (1976) in a personal communication states that regarding the source of ACTH, in teleost fishes it is neither in acidophilic nor in basophilic cells—an obsolete terminology. ACTH is secreted by peptidic cells, but they do not stain with blue or red dyes; they can be demonstrated with the alizarin blue which gives a purple colour, and with the lead haematoxylin which gives a dark blue-black colour. In non-teleosts, their localization seems to be rostral, but no typical staining reaction has yet been determined.

Olivereau studied the role of prolactin in osmoregulation and stimulation of prolactin cells when dopaminergic fibres are destroyed or inhibited and reduction of activity of the same cells when dopaminergic control is stimulated.

Olivereau and Dimovska (1969) identified the cell types in the autotransplanted pituitary gland under the dorsal skin of the eel. The prolactin secreting cells could easily be identified. They had large round nuclei, big nucleoli, and showed almost complete degranulation in 14 out of 15 animals. The corticotrophic cells could be detected along the ramifications of the nervous tissue and these cells were more or less granulated. They showed a subnormal activity. The thyrotrophic cells were angular and well granulated. Their number was less compared to that in the *in situ* pituitary. They could not be found out amongst the few cells. The growth hormone-secreting orangeophilic cells were numerous having dense granulations and variable degree of activity. Amidst these somatotrophic cells there were chromophobic ones which probably corresponded to the gonadotrophic cells which are always poorly differentiated in the control eels. The pars intermedia cells appeared to be quite active and granulated. The neurohypophysis was atrophic having some fibrocytes and numerous pituicytes. The infundibular



recess with ependymal layer could be well visualized and no neurosecretory material was found. The graft was well vascularized.

In the eel only the gonadotrophic cells and one cell type (PAS-positive) of the pars intermedia are completely hypothalamic dependent for their function. Some autonomous activity could be seen in the other cell types when the pituitary loses its connexion with the hypothalamus. The autonomy in the eel seems to be more for the somatotrophic and prolactin cells and perhaps also for the corticotrophic cells than in *Poecilia*. In *Poecilia* the thyrotrophic function is better preserved than in the eel. Pars intermedia of the eel secretes some intermedin.

Oliverieu(1971) studied the histological structure of some endocrine glands in the eel after autotransplantation of the pituitary gland. The number of thyrotrophic cells is always reduced in the transplant and it seems that a basal secretion of this hormone would be sufficient to maintain some thyroidal activity. The thyrotrophic function would appear more independent of a hypothalamic control than in mammals. However, the concept of a TIF (inhibiting factor) does not seem to apply to the eel. The interrenals are similar to those in control eels though there is a reduction in the number of corticotrophic cells in the graft. In the eel, the hypophysio-interrenal axis does not seem to be strictly dependent on a hypothalamic control. There is regression of the gonadotrophic cells in the graft and the organs of Syrski (male gonads) remain poorly differentiated. This indicates the necessity of a hypothalamic stimulation for the maintenance of the gonadotrophic function.

"In grafted eels, the histological picture of the kidney is intermediary between that of intact and hypophysectomized animals. As ovine prolactin partly prevents the renal atrophy induced after hypophysectomy, and as prolactin cells appear very active in all the transplants despite their reduced number, some prolactin secretion probably occurs".

Oliverieu(1971) stated, "the disconnected pituitary of the eel keeps a functional autonomy much more important than in higher vertebrates, the gonadotrophic function being excluded, in agreement with the cytological study of the transplant".

Leatherland(1970) could find ACTH cells in some of the grafts. These cells were either degranulated or totally regressed. Jorgensen(1976) reviewed this subject. Chambolle(1970) grafted the rostral pars distalis ectopically in hypophysectomized *Gambusia*. When there was no ACTH cells in the graft and only LTH cells were present, interrenal cells showed pycnotic nuclei as is noted in hypophysectomized fishes. When ACTH cells were present in the graft, the interrenal cells looked stimulated. In *Poecilia* having ectopically autografted hypophysis cortisol level was reduced to the hypophysectomy level (Hawkins and Ball,1970). Jorgensen(1976) stated, "Apparently, the corticotrophic function of the isolated pars distalis in teleosts, as in anurans, varies with the criteria used to estimate the function".



Ultrastructure of the ectopic hypophysis was studied in *Salmo gairdneri* (Leatherland and Lin, 1976), and *Gillichthys mirabilis* (Nagahama *et al.*, 1974, 1975). Ultrastructure of the rostral pars distalis of *Aphanius dispar* (Ruppel) from hypersaline marshes and freshwater was described by Abraham *et al.* (1977) and Batten *et al.* (1975) described the ultrastructure of the adenohypophysis in the teleost *Poecilia latipinna*. Batten and Ball (1977) described the ultrastructure of the neurohypophysis of the teleost *Poecilia latipinna* in relation to neural control of the adenohypophysial cells. Circadian changes in prolactin cell activity in the pituitary of the teleost *Poecilia latipinna* in fresh-water were described by Batten *et al.* (1976). Follenius (1977) noted inhibition of corticotrophic function in the carp (*Cyprinus carpio* L.) after administration of GABA. Ingleton *et al.* (1977) observed catecholaminergic innervation of the prolactin cells in the teleost *Poecilia latipinna*. Aminergic hypothalamo-hypophysial innervation in *Gambusia sp.* was found by Kah *et al.* (1978). Changes in the prolactin-secreting cells in the cell were observed by Olivereau (1978) after pimozide and parachlorophenylalanine (a brain serotonin depletor). Slijkhuis (1978) studied fanning behaviour and prolactin cell activity in the male threespined stickleback, *Gasterosteus aculeatus*. Changes in external sodium, calcium and magnesium affect prolactin cells, skin and plasma electrolytes of *Gasterosteus aculeatus* (Wendelaar Bonga, 1978).

*In vivo* evidences are there for catechol-aminergic inhibition of prolactin secretion in the teleost *Poecilia latipinna* (Wigham and Ball, 1976). In 1977 Wigham and Ball found effect of environmental salinity changes on the secretory activity of prolactin cells in ocular transplants in the same species. Rostral pars distalis cells of *Gambusia* were studied ultrastructurally in the *in situ* pituitary and after a long-term autotransplantation (5-12 months) by Kah *et al.* (1979). *In situ*, prolactin cells of fish in fresh-water are moderately active. Activity as regards synthesis and release could be found in these cells. Prolactin cells in the long-term grafted pituitary appeared slightly less active. Mitosis, exocytoses, and reinnervation in the grafts could be seen. Fibres made synaptic contact with prolactin cells. This type of innervation existed in the *in situ* gland (type B). The corticotrophs in the grafts were slightly less granulated than those in the *in situ* pituitary. Hypothalamo-hypophysial correlations were also discussed with special reference to those noted in other teleost species.

#### *Central nervous structures which control ACTH release*

In *Ophiocephalus punctatus* Roy (1969) studied the effect of brain lesions on ACTH secretion. Lesions on different parts of the forebrain had no permanent effects on plasma 17-OHCS content. Ablation of the dorsal forebrain significantly increased plasma corticosteroid values one, seven and twentyone days after operation and the values normalised one month after the operation. This dorsal forebrain was the only region in which electrical stimulation significantly reduced plasma corticosteroids from 28.3 mcg/100ml in the sham-stimulated controls to 13.3 mcg in the electrically stimulated. In other regions of the brain electrical



stimulation either increased or did not affect plasma corticosteroids. Ablation of the forebrain including parts of the diencephalon caudal to the optic chiasma significantly reduced corticosteroid levels. Neurons stimulating ACTH secretion take their origin, in the anterior hypothalamus in *Ophiocephalus*. Redgate(1974) observed increased plasma cortisol level after electrical stimulation of the hypothalamus in the carp (*Cyprinus carpio*). Roy(1964) observed increased plasma corticosteroid after forced swimming, surgery, ACTH, pitressin, protopituitrin and histamine injections in *O. punctatus*. Diencephalic neurosecretory extracts of *O. punctatus* and *L. rohita* had CRF action. Pickford *et al.*(1971) studied the stress response in the abundance of circulating leucocytes in the killifish, *Fundulus heteroclitus* with particular reference to the cold-shock sequence and the effects of hypophysectomy. When the intact male fish adapted to 20°C in the sea water, were immersed for three minutes at ca. 1°C, there is transitory coma from which recovery takes place on return to warm temperature. An alternating sequence of leucopaenia and leucocytosis took place with no corresponding changes in the abundance of erythrocytes, in serum chloride or total osmolarity. The typical sequence was leucopaenia at three minutes, leucocytosis at 15 minutes, leucopaenia at 30-60 minutes and leucocytosis at 2 hours. This sequence was followed by gradual return to normal. The major leucocytic cell resembled a lymphocyte. Hypophysectomy abolished or minimized the second leucocytic phase. No change could be found in the earlier phases of the cycle. In the second leucocytic phase serum glucose increased significantly. The authors in the same year studied the role of catecholamines in the stress response and they could not interpret their results in terms of classical mammalian alpha and beta-adrenergic mechanisms. In the same year they studied the role of adrenal cortex and put forward a concluding discussion on the *leucocyte-stress syndrome*. Effect of intraperitoneal injections of ACTH at 2 hours in *Fundulus heteroclitus* depends on the dose. At low doses leucopaenia has been observed and at high doses there is leucocytosis.

The two hour response to cortisol, at 5 µg/g depends on the condition of the fish: leucocytosis in sexually mature or hypophysectomized fish, leucopenia in sexually regressed intact fish. At a physiological dose(0.025µg/g) cortisol elicits a time sequence response in hypophysectomized recipients, that is, initially, the reverse of that observed after cold-shock: three-minute-leucocytosis, 15-minute-leucopenia, and return to normal at 45minutes. Leucocytosis emerges at two hours. Pretreatment with Metopirone for five to six days in the aquarium is not well tolerated. Such treatment effectively blocks all phases of the cold-shock response. Pretreatment for two days was tolerated and in such fish, injection of epinephrine (1µg/g) elicits both three-minute and 30-60-minute-leucopenia, but the leucocytic phases are blocked.

A tentative interpretation of the cold-shock sequence is proposed on the assumption that catecholamines (presumably epinephrine) are leucopenic and that cortisol is leucocytic.



*STH cells*

The STH cells are situated in the proximal pars distalis. In *P. latipinna* these cells are situated in the centro-dorsal part of the proximal pars distalis. In this part they are mixed with TSH cells which are very few (Ball and Baker, 1969). The granules in these acidophils are AB, AF and PAS-negative but in some species they are faintly PAS-positive. Fluorescent antibody to mammalian STH was found to locate specifically in the STH cells of *O. nerka* (McKeown and Van Overbeeke, 1971). Growth hormone is thyrotrophic in *Fundulus heteroclitus* (Giau and Stetson, 1979).

Dubois *et al.* (1974) noted the presence of somatostatin-like activity in the brain, pars distalis, and pars nervosa of the rainbow trout by immunocytochemical method. Vale *et al.* (1976) noted mammalian somatostatin-like activity in extracts of the brains of hagfish, elasmobranch and catfish by radioimmunoassays. Crim *et al.* (1978) studied the comparative endocrinology of piscine hypothalamic hypophysiotrophic peptides—their distribution and activity. Somatostatin-like activity was observed in the extracts of the brain of spiny dogfish and catfish.

Fryer *et al.* (1979) found evidences to suggest that growth hormone (GH) secretion in a teleost, tilapia (*Sarotherodon mossambicus*) was influenced by a somatostatin-like peptide which acts to suppress the release of GH from the pituitary. Komourdjian and Idler (1979) bioassayed highly purified chum salmon pituitary fraction to note their ability to stimulate elongation in hypophysectomized rainbow trout, *Salmo gairdneri*. The sodium—retaining principle (prolactin) was inactive. The most potent one of the active fractions showed a dose response. Specific labelling of somatotrophic cells in pituitary sections by an indirect immunocytofluorescence could be achieved by an antiserum against this fraction. An antiserum against the sodium-retaining principle led to the specific fluorescence of the follicular prolactin cells. No discrimination between acidophil type in pituitary sections from frogs and rats could be done by using any of the two antisera.

Fryer (1979) described the development of a competitive-binding radioreceptor assay for teleost growth hormone (GH) using the binding of <sup>125</sup>I-labelled tilapia GH to particulate membrane fractions prepared from tilapia liver.

Kayes (1979) obtained data to indicate that growth hormone has a controlling influence over nucleic acid metabolism in the bullhead. The response after hypophysectomy and growth hormone replacement therapy is rather quick in the fish and so biochemical and morphometric responses can probably be utilised for assaying teleost growth hormones.

Oliverau and Oliverau (1979) observed estradiol-positive feedback action on gonadotrophic cells in fresh-water male silver eels. Injection of estradiol-17 $\beta$  for twenty and forty days showed hypertrophy, increased vacuolar content, and glycogen depletion of the liver. The plasma is strongly opalescent probably due to the presence of vitellogenin. Plasma sodium is slightly decreased and calcium



is considerably increased. These changes regress partly when the eels are sacrificed 40 days after the end of the treatment. Even at this period the values do not normalize. A few unusual oocytes have been found in the organs of Syrski. Pituitary prolactin (PRL) and growth hormone (STH)-secreting cells are stimulated. Enlarged gonadotrophs are differentiated having plenty of coarse glycoprotein granules. TSH cells appear to be stimulated after a long-term treatment. MSH cells are less active and PAS + cells of the pars intermedia are degranulated. These responses disappear or reduce greatly after 40 days without treatment. Gonadotrophs are significantly smaller and less granulated. Estradiol (E2) acts on GTH cells and stimulates synthesis of hormones by a positive feedback.

Benjamin(1978) noted cytological changes in prolactin, ACTH and growth hormone cells of the pituitary gland of *Pungitius pungitius* L. in response to increased environmental salinities. Intercellular cystic formations were found among the prolactin cells which obliterated the rostral pars distalis at 21 days. The prolactin cells diminished in number. All these events indicated decreased secretory activity of the prolactin cells. Nuclear diameters in prolactin, ACTH, and growth hormone cells were found to be decreased. Degranulation response was observed in the growth hormone cells of the animals from the 21-day sea water group.

There are similarities between growth hormone and prolactin regarding amino acid composition, immunochemistry, and activity in the rat tibia assay. Teleosts can readily distinguish between *Tilapia* growth hormone and *Tilapia* prolactin (Clarke, Farmer, and Hartwell, 1977).

Hall and Chadwick(1978) studied the control of prolactin and growth hormone secretion in the eel *Anguilla anguilla*. When fresh water eels were placed in seawater, prolactin and growth hormone came to a low level initially but after eight weeks they returned to the original values. Difference in the release of prolactin *in vitro* from the whole glands could not be observed compared to the release of the hormone from the pituitary fragments consisting of rostral pars distalis only. Prolactin and growth hormone release *in vitro* was directly proportional to the amount of hypothalamic extract added. There was decrease in hypothalamic prolactin and growth hormone stimulating activity during adaptation to seawater. Pituitaries of seawater-adapted eels could respond to hypothalamic extract from freshwater eels.

#### GTH (Gonadotrophic cells) (fig. 10.6)

These cells are situated in the ventral parts of the proximal pars distalis and Olivereau(1972) found them to spread into the rostral pars distalis during sexual maturity in various teleosts e.g. the eel, salmon and trout. The fish has a single gonadotrophin with FSH and LH-like properties (Burzawa-Gerard and Fontaine,1972; Sundararaj *et al.*,1972; Chester Jones *et al.*,1973). FSH and LH cells could be separately found by Knowles and Vollrath(1966) by electron



microscopy. These differentiations of gonadotrophs even to more different cellular types may be the different stages of cellular activity in a single cell type.

The gonadotrophs are situated in the proximal pars distalis. The rostral zone may be invaded by these cells or an irregular layer may be formed by them around the neuro-intermediate lobe. The glycoprotein granules in these cells are AF+, PAS+, and AB+. The cells also contain a few globules (1 to 2  $\mu$ m in diameter) (Stahl,1963; Sage and Bern,1971; Rao,1969,1972; Rai,1972; Schreibman *et al.*,1973; Tsuneki and Ichikawa,1973).

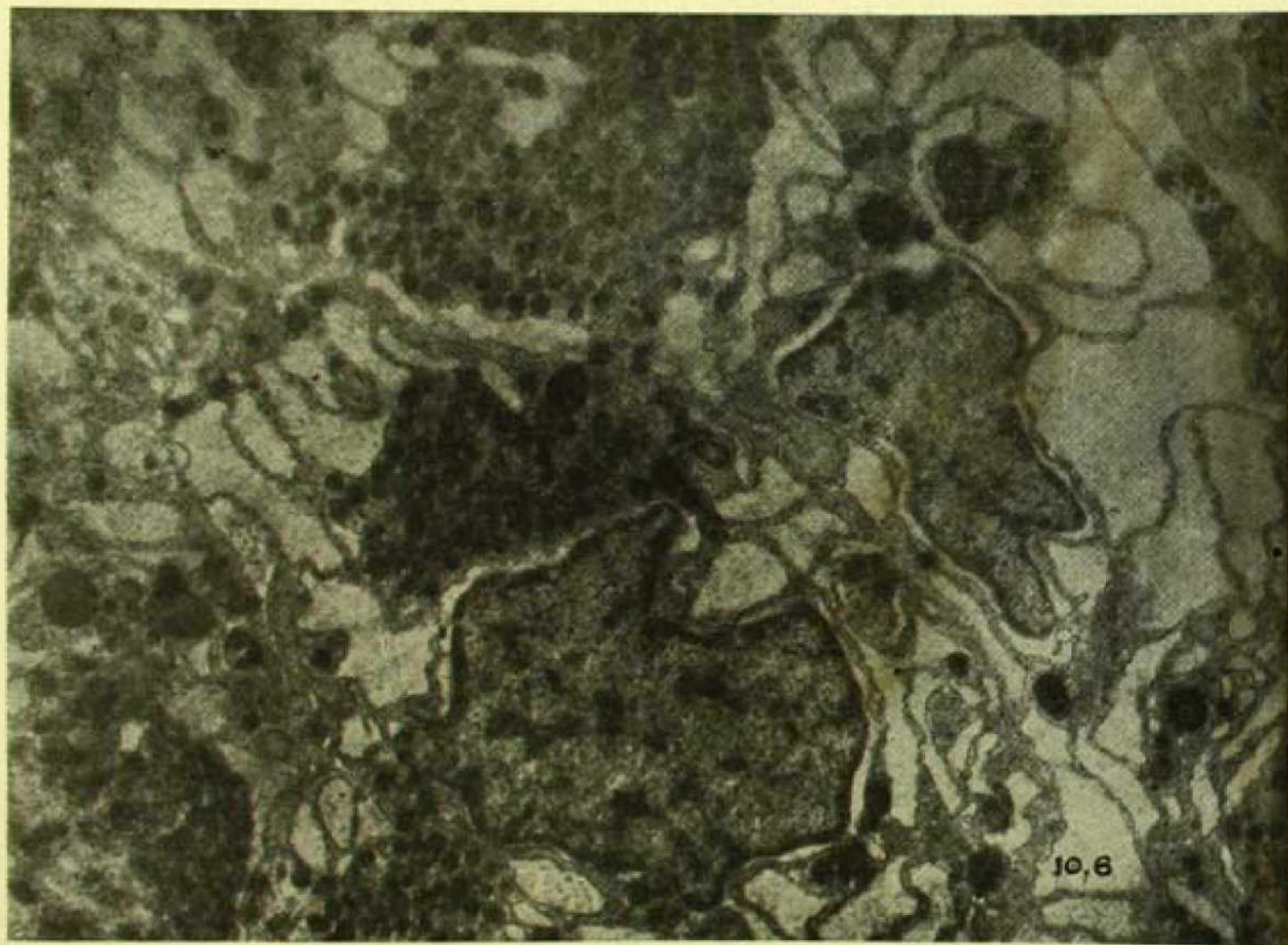


Fig. 10.6. Granulated cells in the proximal pars distalis of the teleost, *Chasmichthys dolichognathus*. These cells are basophilic at LM level. They may be gonadotrophic; however, the possibility that they are thyrotrophic cannot be excluded. Courtesy of Professor Kobayashi and Dr. Tsuneki (1977).

The gonadotrophic cells may be either of one type (Follenius and Porte,1960; Olivereau and Ball,1964; Schreibman,1964; Matheij,1970; Rao,1972; Schreibman *et al.*,1973; Schreibman and Margolis-Kazan,1979) or of two types. Some species may contain two types of PAS+ and AF+ glycoprotein cells which are not thyrotrophic. During reproductive cycle they behave differently. In the cell they have characteristic staining reactions with Herlant's tetrachrome (Olivereau and



Herlant, 1960) or lead haematoxylin (Olivereau, 1967). The plurality of the cells has also been identified by electron microscopy (Knowles and Vollrath, 1966). The similar plurality of gonadotrophic cells has also been found in the goldfish (Olivereau, 1962), *Lepomis* (Simon, 1971), the perch (Dimovska, 1970, 1972), *Boops salpa* (Michele, 1973), Salmonidae (McKeown and Leatherland, 1973), Atlantic salmon (Olivereau, 1976), *Gasterosteus aculeatus* (Slijkhuis, 1978), and the loach, *Misgurnus anguillicaudatus* (Ueda and Takahashi, 1980).

One type of gonadotrophin has been isolated from carp, salmon or sturgeon pituitaries (Fontaine and Gerard, 1963; Yamazaki and Donaldson, 1968; Burzawa-Gerard and Fontaine, 1972). A single protein molecule in piscine gonadotrophins can stimulate all gonadal functions (Donaldson, 1973). Idler *et al.*, (1975) noted the presence of two gonadotrophins in salmon. Farmer and Papkoff (1977) found indications for the existence of two gonadotrophins in the tilapia fishes. Idler and Campbell (1980) showed that carbohydrate-rich gonadotrophin stimulates vitellogenin synthesis but the carbohydrate-poor gonadotrophin does not, and "support a hypothesis of action by two gonadotrophins in regulation of teleost vitellogenesis". Burzawa-Gerard and Dufour (1980) studied the specificity of antigenic determinants of carp gonadotrophin  $\alpha$  and  $\beta$  subunits. "A fish gonadotrophin  $\beta$  subunit (c-GTH $\beta$ ) appears more closely related to mammalian LH $\beta$  than to FSH $\beta$  and exhibits an intermediate relatedness with TSH $\beta$ " (from Ball and Batten, 1980).

Hyder *et al.*, (1979) studied the effects of tilapia partially purified pituitary gonadotrophic fractions on the testes of methallibure (gonadotrophic inhibitor)-treated *Sarotherodon spirulus* (= *Tilapia nigra*). They used tilapia pituitary homogenate (TPH), tilapia pituitary glycoprotein fraction (T-GTN), tilapia pituitary glycoprotein CM-cellulose fraction 1 (T-CM-1) or fraction 2 (T-CM-2). TPH and all the fractions had some gonadotrophic activity. The most comprehensive effects were found with T-GTN and T-CM-1. T-CM-2 was effective at a higher dose. Most of the gonadotrophic activity was concentrated in the T-CM-1 fraction. The same purification method in higher vertebrates concentrates FSH and LH in the CM-1 and CM-2 fractions respectively. T-CM-2 fraction containing LH did not predominantly stimulate interstitial tissue activity in this study. FSH is contained in T-CM-1 fraction though Burzawa-Gerard and Fontaine (1972) considered the existence of FSH-like piscine gonadotrophin to be highly dubious. Ng and Idler (1979) noted two types of gonadotrophins from both American plaice and winter flounder pituitaries. They also noted two types of gonadotrophins from both salmon and carp pituitaries (Con A1 hormone is a preparation which is unadsorbed on concanavalin A-Sepharose and this has been purified by processes such as gel filtration and ion-exchange chromatography. Con A2 hormone is adsorbed on the immobilized lectin and purified by a similar procedure).

Schreibman (1962, 1964) and Schreibman and Charipper (1962) observed in *Xiphophorus maculatus* that the peripheral basophils of the meso-adenohypophysis did not appear before six or eight weeks, when the gonads also start developing.



These cells increased in size and number in gravid females. Hypertrophy of the peripheral meso-adenohypophysial basophils and hyalinization of the cytoplasm having red or orange staining bodies were encountered in ageing females. In castrated males hypertrophy, degranulation and vacuolation were noted in these cells. A sex-linked gene, P, determines the sexual maturity occurring in the platyfish *Xiphophorus maculatus* (Kallman and Schreibman, 1973; Schreibman and Kallman, 1977, 1978; Kallman and Borkoski, 1978). The gonadotrophic zone forms the external border of the caudal pars distalis (CPD) and its width is formed by several cells. Somatotrophs and thyrotrophs are contained in the central part of the CPD. Rostral pars distalis contains prolactin secreting cells and corticotrophs. MSH cells and cells with possible unknown product are situated in the pars intermedia.

Schreibman and Margolis-Kazan (1979) used immunoperoxidase method for identification of gonadotrophin (GtH)-and thyrotrophin (TSH)-producing cells in the CPD of the pituitary gland of mature platyfish, *Xiphophorus maculatus*. Other workers (McKeown and van Overbeeke, 1971; Billard *et al.*, 1971; Goos *et al.*, 1976; Ekengren *et al.*, 1978) used immunofluorescence technique for localization of GtH-cells in different teleosts. The first report of the use of peroxidase method for localization of immunoreactive glycoprotein hormones in the fish is that of Margolis-Kazan *et al.* (1978). According to Schreibman and Margolis-Kazan (1979) the peroxidase method is more desirable than the immunofluorescence technique. Antiserum to carp GtH (anti-cGtH) showed highest degree of specificity when compared to trout and salmon preparations produced against intact GtH molecule. Anti-cGtH- $\alpha$  cross-reacted with thyrotrophs and gonadotrophs whereas, anti-cGtH- $\beta$  showed immunoreactivity with gonadotrophs and not with thyrotrophs. Anti-human TSH cross-reacted only with thyrotrophs. This report is "the first to show that the  $\alpha$ -subunit of carp GtH is a chain common to both TSH and GtH in fish. It is also the first demonstration of antigenic similarity between human and fish TSH". Anti-cGtH- $\beta$  cross-reacted also with PAS+ cells in the pars intermedia.

Truscott *et al.* (1978) observed the effects of gonadotrophins and ACTH on plasmatic steroids of the catfish, *Heteropneustes fossilis* (Bloch). Plasma cortisol and testosterone levels increase in the gravid catfish after gonadotrophin administration. They may play a role in oocyte maturation either singly or synergistically.

Cook *et al.* (1980) observed that cortisol may play a physiological role in ovulation in the goldfish. A new method was developed by Sangalang *et al.* (1980) to determine fish plasma cortisol by radioimmunoassay.

Stimulation of gonadotrophin secretion occurs after castration in male rainbow trout (Billard *et al.*, 1977). After castration the rise was four times the initial value in May and September. It increased twofold in October and sevenfold in December.

Schreibman and Halpern (1980) demonstrated neurophysin and arginine vasotocin by immunocytochemical methods in the brain and pituitary gland of the platyfish, *Xiphophorus maculatus*. Immunologically they are distinct entities



as shown by control absorption procedures. "The two appear to be elaborated and stored in similar locations and follow comparable routes of transport between the nucleus preopticus and the pituitary gland."

Sex steroid-concentrating cells were located in the ventral telencephalon, preoptic area, lateral tuberal nucleus, nucleus of the lateral recess of the third ventricle, and caudal portion of the posterior periventricular nucleus in the teleost *Macropodus opercularis* (paradise fish : male). Many labelled cells were also situated in the caudal pars distalis of the pituitary. Steroid-retaining cells were not found in the mesencephalon, rhombencephalon, or anterior spinal cord (Davis *et al.*, 1977).

Kim *et al.* (1978) described the topography of estrogen target cells in the forebrain of goldfish, *Carassius auratus*. Estrogen and androgen target cells were noted by Kim *et al.* (1978) in the brain of fishes, reptiles and birds.

Oliveriau and Oliveriau (1979) studied the effect of estradiol-17 $\beta$  (E<sub>2</sub>) on the cytology of the liver, gonads and pituitary, and on plasma electrolytes in the female freshwater eel. Treated female eels appeared paler and secreted more mucus than controls. Blood plasma became strongly opalescent indicating vitellogenin synthesis. This synthesis occurred in the hypertrophied liver which had increased vacuolization (lipid material) and glycogen depletion. Plasma sodium was lowered with increase in calcium levels. The gonosomatic index increased. Oocytes were enlarged but the incorporation of vitellogenin remained discrete. In control eels the gonadotrophs (GTH cells) are small and scarcely visible in the pituitary. After E<sub>2</sub> administration, GTH cells are hypertrophied and contain numerous glycoprotein granules. By a positive feedback action, E<sub>2</sub> may act on the pituitary and/or hypothalamus to induce GTH synthesis. GTH release seemed to be very limited as was evidenced by the ovarian response. "The differentiation of GTH cells in eels treated with fish pituitary extracts is most probably due to secretion of E<sub>2</sub> by the ovary, which reacts on the pituitary. Various hypotheses are considered to explain the low GTH release." Stimulation of thyrotrophs, somatotrophs and prolactin cells of the pituitary takes place. Pars intermedia MSH and PAS+ cells appeared less active. Antidopaminergic effect of E<sub>2</sub> is possible. E<sub>2</sub> administration is a simple and economic technique to induce synthesis of GTH.

Oliveriau and Chambolle (1979) studied the ultrastructure of gonadotrophs in the eel following estradiol (E<sub>2</sub>) treatment. Few poorly differentiated gonadotrophs have been noted in the male and female silver eels, in fresh water or sea water. Estradiol treatment leads to their development and hyperplasia. The Golgi complex is well developed and the secretory granules (200-500nm) are numerous. Dilated cisternae and large globules (1.2-2.2  $\mu$ m) are also observed. Probably the large globules are lysosomes. No exocytosis could be found. Because no macroscopic effect is discernible on the gonad, the release of the synthesized hormone remains questionable. Several hypotheses have been offered to explain these



data: (a) the synthesized gonadotrophin is not released. It may be due to an insufficient secretion of LH-RH in the hypothalamus. (b) The gonad is refractory to release gonadotrophin or its action is inhibited by excess of circulating  $E_2$ . (c) Two separate gonadotrophins control vitellogenesis and maturation in teleosts. Gonadal incorporation of the yolk is not stimulated by the glycoprotein preparation and  $E_2$  treatment. A nonglycoprotein fraction induces this incorporation. One type of gonadotroph shows poor reactions for glycoproteins. This type is not observed after  $E_2$  treatment in the eel. Oivereau and Chambolle (1979) further stated that  $E_2$  exerted a positive feedback on the gonadotrophic activity of immature eels without inducing sexual maturation.

Prasada Rao *et al.* (1979) noted two divisions of the nucleus preopticus (NPO) in the catfish (*Clarias batrachus*). One vertical neuronal group is situated near the preoptic recess and this has been identified as NPO-paraventricularis (NPO-P). The other division, NPO-supraopticus (NPO-S) is situated horizontally above the optic chiasma. Bridge cells are noted in between the two divisions. Cytoarchitectonically, the NPO-P can be divided into four subdivisions, and the NPO-S into three subdivisions. Significant stimulatory changes have been noted only in the medial, lateral and postero-dorsal subdivisions of the NPO-P after ovariectomy (40 days), while no remarkable change occurred in the pars paraventricularis. The gonadotrophs undergo hypertrophy and hyperplasia. Regressive changes occurred in the same neuronal groups after estradiol benzoate (EB). The gonadotrophs are also regressed. Significant changes were not found in the subdivisions of the NPO-S (pars ventralis, pars medialis, and pars lateralis) after ovariectomy or administration of EB.

Colombo *et al.* (1979) concluded, "teleosts display a very versatile use of gonadal steroids in the integration of reproductive processes. From intragonadal targets, steroid control has been extended apparently to peripheral sexual organs and brain centers and further adapted to mediate sexual communications. The complexity of the regulatory circuits channelled through the gonads of fish is certainly not less impressive than that of land-living vertebrates and its appreciation should be indeed a good start for future research."

#### Control of gonadotrophin release

Goos and Murathanoglu (1977) noted GnRH in the forebrain of *Salmo* immunocytochemically. Follenius and Dubois (1977, 1978) localized immunocytoologically  $\alpha$ -endorphin-like peptides in the neurons of nucleus lateralis tuberculi and neurohypophysis of *Carassius auratus* and *Cyprinus carpio*. Gonadotrophin secretion in teleosts is stimulated by synthetic LHRH (Breton and Weil, 1973; Kaul and Voilrath, 1974; Crim *et al.*, 1976; Lam *et al.*, 1976; Ekengren *et al.*, 1978). Young and Ball (1980) studied the effects of LHRH on the ultrastructure of the gonadotrophic cells of the pituitary of *P. latipinna* *in vivo* and *in vitro*. The authors concluded that gonadotrophic cells of *P. latipinna* are controlled by LHRH-like peptide and "secretory material may be released directly from the RER without the involvement of the Golgi apparatus" (from Ball and Batten, 1980).



Van Oordt and Ekengren(1978) found small perikarya with immunoreactive cytoplasm and axon swellings in the area dorsalis partis medialis (ADPM) of the telencephalon in *So gairdneri*. Numerous perikarya proceed diffusely in the lateral walls of the diencephalon towards the pituitary stalk. The neurohypophysis also contains these fibres. The authors concluded that besides the cells of the nucleus lateralis tuberis, neurons in the ADPM are involved in the production of gonadotrophin releasing hormone (GRH). Thus the GTH-cells of some teleosts have a double neuro-endocrine control. The other method of control is by direct innervation of GTH-cells by two different types\* of neurons as has been observed in some species.

Peter and Paulencu(1980) observed the preoptic region to be involved in gonadotrophin release-inhibition in goldfish, *Carassius auratus*. In sexually mature female and male goldfish various hypothalamic and preoptic regions were lesioned. Ovulation and increase in serum gonadotrophin levels occurred after destruction of the pituitary stalk, and lateral anterior hypothalamic tract areas. The same results were obtained after destruction of the entire preoptic region or a major part of the anterior nucleus preopticus periventricularis. Lesions in other locations were ineffective. "The results indicate that a gonadotrophin release—inhibitory factor (GRIF) probably originates in the anterior preoptic region, and reaches the pituitary via pathways in the lateral preoptic and lateral anterior hypothalamic regions, and the pituitary stalk. The preovulatory surge of gonadotrophin secretion may be regulated by release from inhibition exerted by GRIF in goldfish."

Batten and Ball(1977) observed that a single typeB fibre and 5 different subtypes of typeA fibres could innervate the pituitary in *Poecilia latipinna*. Peter and his associates (1970-1977) could ascribe the control of thyrotrophin, gonadotrophin, and ACTH secretion to the nucleus lateralis tuberis of the goldfish. ACTH secretion is also controlled by the nucleus preopticus.

Batten, Ingleton and Ball(1979) conducted ultrastructural and formaldehyde-fluorescence studies on the hypothalamus of *Poecilia latipinna*. In the nucleus preopticus two different types of neurons could be identified ultrastructurally. TypeI neurons were large. They could be differentiated from the smaller type2 neurons by the appearance (although not size) of the dense-cored vesicles (DCV). TypeI neurons possess more extensive rough endoplasmic reticulum (RER). Both the types had axonal processes and only type2 neurons had ciliated cerebrospinal fluid-contacting dendrites terminating in the ventricle. The nucleus lateralis tuberis could be divided into six areas. Several distinct cell types could be identified ultrastructurally in these areas. The pars posterioris of the nucleus lateralis tuberis contained neurons with 75-85nm DCV. The pars lateralis contained large cell bodies with extensive arrays of distended RER and plenty of DCV (150nm on an average). Scattered cell bodies comprised the pars rostralis. They were similar to, but smaller than the previous group. CSF-contacting neurons were also noted in the nucleus lateralis tuberis (NLT). TypeB(aminergic) fibres innervated the nucleus preopticus (NPO) and NLT. The nucleus recessus



posterioris (NRP) and nucleus recessus lateralis (NRL) possessed a single type of neuron containing plenty of DCV (80nm). This cell type showed long axonal processes which proceeded towards the NLT and the ciliated apical processes projected into the ventricle. Strong monoamine fluorescence by the Falck-Hillarp method could be observed *only* in the NRP and NRL of all the nuclei examined by the authors. Several fluorescent tracts ran between the NRP and NRL and also towards the NLT and the pituitary. The authors concluded that these nuclei are the main source of the hypothalamic and pituitary *typeB* nerve endings. They can be observed as fluorescent varicosities with the Falck-Hillarp method. "Evidence for NPO and NLT cell bodies being the origin of the hypophysial peptidergic *typeA* fibres is discussed, together with indications that these nuclei might be involved in control of adeno-hypophysial activity". In the pituitary of *Poecilia latipinna* Batten *et al.* (1979) found prominent and plenty of fluorescent varicosities among the prolactin cells in the rostral pars distalis. Vertical band of diffuse fluorescence could be found behind the rostral pars distalis and this corresponded to the double basement membrane separating the neurohypophysis from the ACTH cells. The authors thought this fluorescent band to correspond to the *typeB* fibre endings which had synapse on the basement membrane. Some varicosities were noted in the pars intermedia (PI 2) (lead-haematoxylin +) cell. In the proximal pars distalis and the neurohypophysis the varicosities and the diffuse fluorescence corresponded to the *typeB* nerve endings. Increase in the induced fluorescence by L-DOPA and Pargyline was noted by the authors in the varicosities. Induced fluorescence could be noted by them in PAS+PI 1 cells of the pars intermedia and sometimes in the gonadotrophs of the proximal pars distalis. With 6-OHDA treatment reduction in the intensity of fluorescent varicosities was noted in the prolactin cells, ACTH cell area, and PI 1 cells. NRP and NRL had slight reduction of fluorescence.

Batten and Ball (1980) described the ultrastructure of hypophysiotrophic centres in the hypothalamus of *Poecilia latipinna*. *TypeB* fibres are aminergic and contain 80nm to 100nm dense-cored vesicles (DCV). *TypeA* fibres are peptidergic and contain 100nm to 200nm neurosecretory granules. The authors in 1977 noted different subtypes of *typeA* fibres in *P. latipinna*. Ultrastructurally two distinct cell types have been detected in the nucleus preopticus. The granules in both these types are on an average 140nm in diameter. Pituitary innervation by *typeA* fibres is made by these cell types. The neurosecretory granules in the cells of the pars lateralis of nucleus lateralis tuberculi are similar to those noted in some *typeA* fibres. Though changes in these cells could be correlated with gonadotrophic functions previously by Zambrano (1971), recent studies by Batten and Ball (1980) did not demonstrate such correlation in ovariectomized *P. latipinna*. In several species NLT cell types have DCV similar to those noted in *typeB* fibres. Few cells located in the pars posterioris of NLT of *P. latipinna* have DCV of this size. *TypeB* innervation in this species comes from aminergic neurons of the nucleus recessus posterioris. They contain plenty of DCV and long granulated processes proceed towards the pituitary.



### TSH cells

The thyrotrophs have greater affinity for AF and AB (pH 0.2) than the gonadotrophs (Ball and Baker, 1969; Mattheij, 1968; Mattheij *et al.*, 1971). In the rostral zone cells (glycoprotein granulations) are PAS-positive, AF-positive and AB-positive. These cells secrete TSH in Anguillidae (Olivereau, 1963) and various Salmonidae. (Olivereau *et al.*, 1964; Baker, 1969; Olivereau, 1972). In other species they are situated in the proximal zone. They are dorsal in the cyprinodonts (Olivereau and Ball, 1964; Schreibman, 1964). In *Mugil* these cells form a compact cluster in between the two subdivisions of the pars distalis (Olivereau, 1968; Leray, 1968). In the carp, an antibody prepared from a TSH-rich carp pituitary extract shows a positive reaction with a cell type thought to be thyrotrophic (Billard *et al.*, 1971).

Holmes and Ball, (1974) summarized, "despite species variation it is possible to generalise and state that the teleost hypothalamus, unlike that of mammals, does not seem to stimulate TSH secretion, which is either autonomous or is primarily influenced by a TIF. It is still possible that a TRF may also be elaborated, and that it may be found to predominate in some teleosts."

Thyroid activity is greatly reduced in *Fundulus heteroclitus* after hypophysectomy (Pickford, 1953; Pickford and Atz, 1957; Gorbman, 1969). Pituitary transplants secrete thyroid stimulating hormone (Ball *et al.*, 1963; Ball *et al.*, 1972; Peter, 1973). Injury to the nucleus lateralis tuberis (pars anterior or pars posterior) increased thyroid activity and this indicates an inhibitory hypothalamic control of TSH secretion in teleosts. By hypophysectomy and pituitary autotransplantation in *Fundulus heteroclitus* Grau and Steison (1977) concluded that for maintenance of normal  $T_4$  levels, pituitary stimulation is required and inhibitory hypothalamic control exists for pituitary thyrotrophin release.

### *Pars intermedia* (figs. 10.7 & 10.8)

Two cell types are present. They are type I and Type II (Benjamin, 1973). Olivereau distinguishes them by the staining reactions: Lead haematoxylin-positive cell (Olivereau, 1970) and having large (250 to 400nm) granules is one type. The other type of cell is PAS-positive with smaller granules (120 to 200nm) (Knowles and Vollrath, 1966). Lead haematoxylin-positive cells in the eel secrete MSH (Olivereau, 1971, 1972; Baker, 1972; Fremberg and Olivereau, 1973). These cells are responsible for MSH secretion in the Tench (Romain, 1974). MSH secretion has been located in the lead haematoxylin-positive cells by immunofluorescence in the stickleback (Follenius and Dubois, 1974) in the presence of an anti- $\beta$ -MSH antibody and in the perch (Follenius and Dubois, 1976).

Fontaine and Olivereau (1975) said, "the PAS-positive cells form a distinct population whose role is unknown, but in the eel their intense hyperplasia in deionized water (Olivereau, 1967) and their decreased activity in sea water (Olivereau, 1969) suggest an osmoregulatory function".



Salmonidae and *Chasmichthys dolichognathus* (Tsuneki and Ichikawa, 1973) have only lead haematoxylin-positive cells. "Since 70 to 90% of the Salmon's MSH activity is present in the neuro-intermediate complex (Fontaine-Bertrand *et al.*, (1969), it may be supposed that these cells secrete MSH".

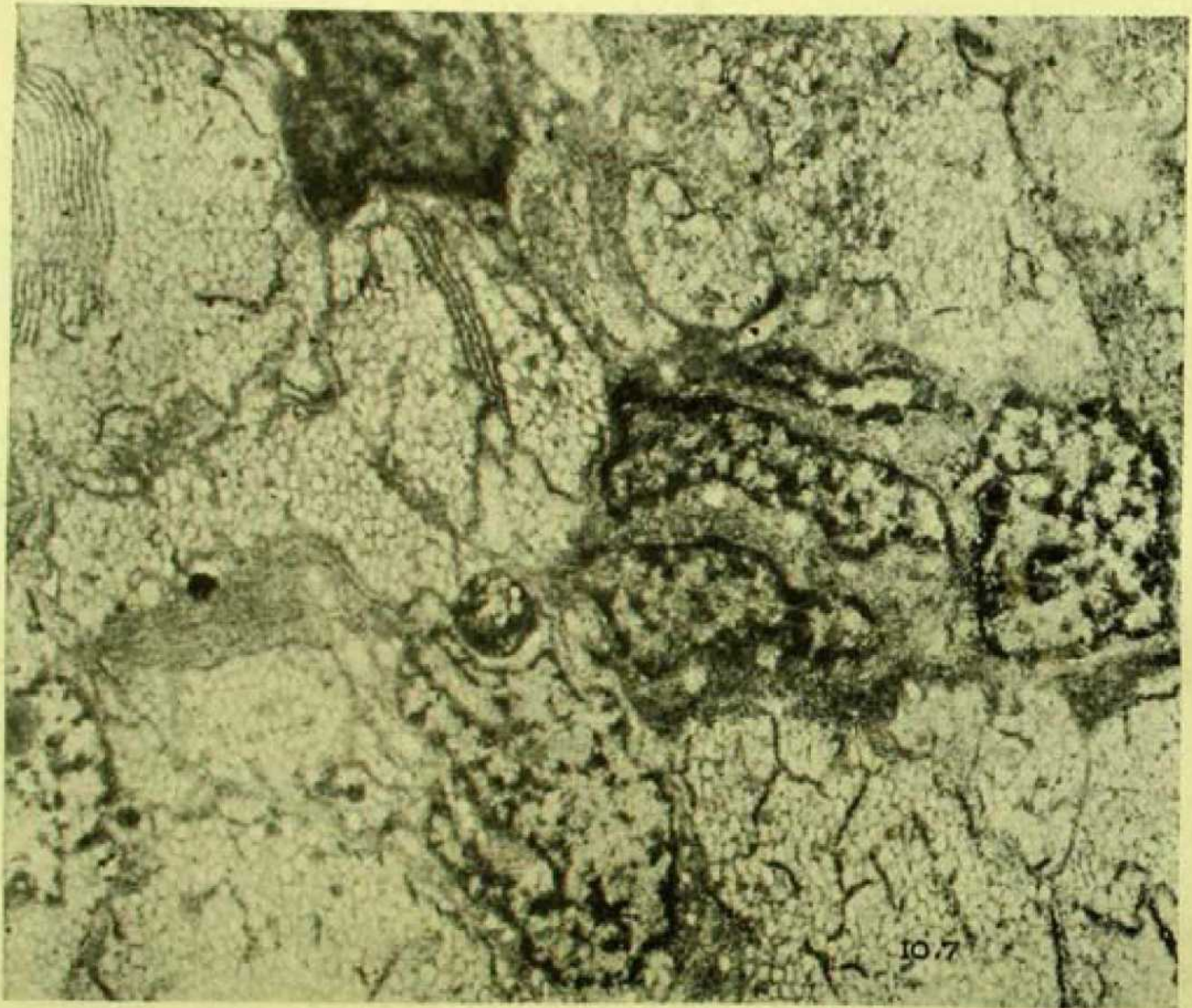


Fig. 10.7. Pars intermedia of the teleost, *Chasmichthys dolichognathus*. Secretory granules probably related to MSH are lucent in this picture.  
Courtesy of Professor Kobayashi and Dr. Tsuneki (1977).

Malo-Michele (1977) noted PbH + and PAS + cells in the pars intermedia of the teleost *Boops slapa*. Both cell types are stimulated by black background adaptation. PAS + cells regressed on a white background. Strong hyperplasia and hypertrophy of PAS + cells was noted after black background adaptation combined with permanent illumination. Both cell types are activated by permanent illumination.

A simple bioassay method for the teleost melanin-concentrating hormone (MCH) has been described by Rance and Baker (1979). They used this assay and also the *Anolis* bioassay for MSH. It compares the relative concentrations of MSH and MCH in the pituitary of various teleosts and their distribution pattern



after polyacrylamide gel electrophoresis. Neurointermediate lobes from trout were cultured and the effects of cold, cycloheximide, EGTA and high potassium ion concentration were studied with respect to MSH and MCH secretion. MSH

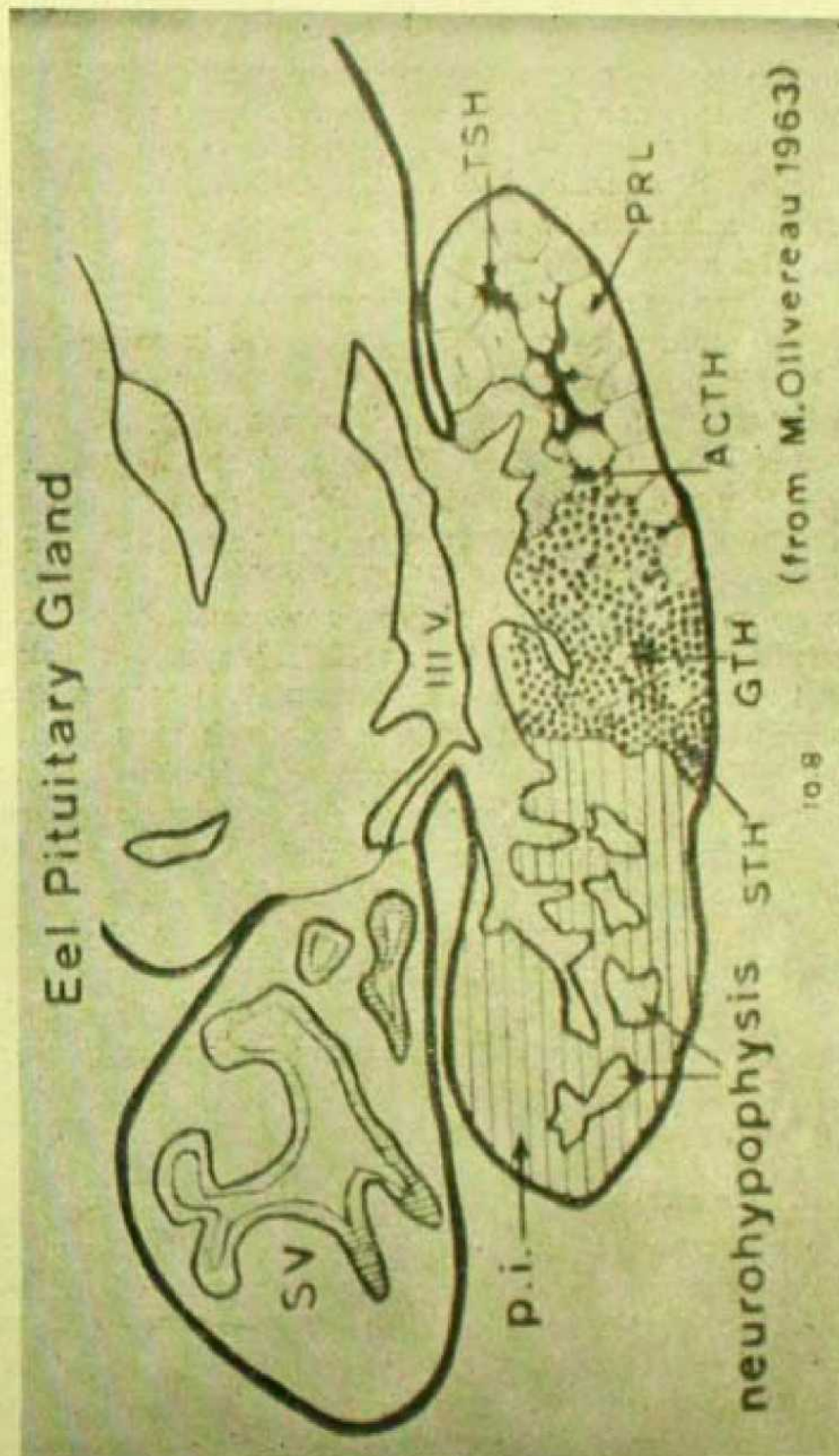


Fig. 10.8. To show the pars intermedia, neurohypophysis and arrangement of the cell types in the rostral pars distalis of the eel pituitary gland. (SV), saccus vasculosus; (Pi), pars intermedia; (III V), third ventricle; (STH), somatotrophic cells (GTH), gonadotrophic cells; (ACTH), corticotrophic cells; (PRL), prolactin cells; (TSH), thyrotrophic cells.—From Olivereau (1963).  
Courtesy of Professor M. Olivereau and Editors, Gen. Comp. Endocrinol.



release was inhibited by EGTA and high potassium ion. MSH synthesis appeared to be reduced by cold and cycloheximide. Effects on MSH were predictable and consistent. MCH response was erratic and unpredictable. *In vitro* synthesis of MSH was found but there was no evidence for MCH synthesis. Trout hypothalamus and pituitary equally contain MCH. Hypothalamic MCH concentration varies with the background colour. The authors conclude that MCH is a hypothalamic secretion. It is stored and released by the neurohypophysis.

After injections of parachlorophenylalanine (pCPA) in the eel, Olivereau (1978) found the animals to be pale, with a low melanophore index. In the pituitary gland, the granules tend to accumulate in the basal part of the MSH cells and in the perinuclear area. Cells appeared smaller with a decreased nuclear area. The neurosecretory material was found to be reduced in the neurohypophysis. Injections of 5-hydroxytryptophan on the other hand, induced a strong darkening as reported in some amphibian species and in one lacertilian species. Olivereau (1978) thought that 5-hydroxytryptophan has a stimulatory influence on MSH-release and possibly its synthesis in the eel and other lower vertebrates.

Pimozide is a specific blocker of dopaminergic receptors. It was injected in fresh water (FW) eels or eels acclimated to sea water (SW). Melanophore index increases in FW and in 1 month-SW injected eels. Olivereau (1978) observed total or subtotal degranulation of the lead-haematoxylin + cells in the pars intermedia in all the treated fish. These cells are  $\alpha$ -MSH-secreting cells. The nuclear area of MSH cell is increased, nucleoli are larger, and the endoplasmic reticulum is well developed. There is no difference in the intensity of the response in FW and SW eels. Intensity of the response is not increased with higher dose. With immunofluorescence and immunoenzymologic techniques, rapid release of pituitary  $\alpha$ -MSH has been observed. No effect on the second cell type of the pars intermedia (PAS+cell) has been noted. The neurosecretory material in the neurohypophysis has been found often to be reduced. "These results suggest that the hypothalamic inhibitory control of MSH release and synthesis is mediated through dopaminergic fibres in the eel, but other factors cannot be ignored in this regulation."

Olivereau and Olivereau (1979) studied the effect of serotonin on prolactin (PRL) and MSH-secreting cells in the eel and compared with the effect of 5-hydroxytryptophan. Parachlorophenylalanine appeared to reduce the release of prolactin (PRL) and melanocyte-stimulating hormone (MSH) in the eel. Therefore a stimulating serotonergic control of these adeno-hypophysial secretions was suspected. Eels were injected with serotonin (5-HT) and compared with eels injected with the precursor of serotonin, 5-hydroxytryptophan (5-HTP). In both the cases, a darkening of the skin was observed. The response was more rapid and intense with 5-HTP than with 5-HT. Degranulation of MSH cell was more complete in 5-HTP-treated eels. An unexplained dilatation of swim-bladder simultaneously occurred in this group but it was not detected after 5-HT



treatment. PRL cells were found to be stimulated in both cases with increase of nuclear areas of PRL and MSH cells. "These results suggest that a serotonergic pathway stimulates PRL and MSH release in the eel. An antagonism between the serotonergic system and the dopaminergic system previously demonstrated in the same species seems apparent, but the interaction of other organs or factors, such as the pineal, are considered". Serotonin promotes MSH release in a lizard, *Anolis carolinensis*, which seems to be more sensitive than the eel. The skin darkening starts after two minutes and lasts for about fifteen minutes at a dose of 3 to 10  $\mu\text{g/g}$  (Thornton and Geschwind, 1975). It acts directly on the pars intermedia of *Anolis*. In the eel 5-HTP is more potent (rapid response) than 5-HT. Darkening response needs the presence of the pituitary as it does not happen in hypophysectomized eels. It cannot be ascertained whether fish brain contains  $\alpha$ -MSH-like peptide. Even if it is present as in the rat (hypothalamus, thalamus and other parts of the brain) or in *Rana esculenta* (diencephalon, telencephalon and rhombencephalon), it has practically no role in the control of skin melanophores. Brain MSH is not released by a serotonergic pathway because 5-HT and 5-HTP have no darkening effect on the MI of hypophysectomized eels. The melanophore hormones,  $\alpha$ - and  $\beta$ -MSH pass the CSF-blood barrier in the killifish, *Fundulus heteroclitus* with difficulty, if at all (Knight, Knight, and Pickford, 1978). Brain  $\alpha$ -MSH is not able to enter peripheral blood vessels and so it is prevented from acting on skin melanophores. In the rat  $\alpha$ -MSH can pass through the blood brain barrier (Pelletier *et al.* 1975). Olivereau *et al.* (1976) demonstrated the presence of  $\alpha$ -MSH in PbH granules of the pars intermedia cells (Immunoenzymological technique). The peptide which reacts with antisera against 1-24 ACTH and 17-39 ACTH is also located in the PbH+ granules. The part played by pars intermedia associated—ACTH is not clear at present. Corticoliberin stimulates ACTH release from the pars distalis but it has no action on ACTH in the pars intermedia. 5-HT has no action on pars distalis ACTH but it releases ACTH from dispersed rat pars intermedia cells (Kraicer and Morris, 1976). 5-HT and 5-HTP degranulate PbH+ cells indicating release of  $\alpha$ -MSH and ACTH-like peptide in the eel.

Olivereau and Chambolle (1979) studied the ultrastructure of the MSH-secreting cells in 5-HTP treated eels. Melanodispersion occurs after injection of 5-HTP. MSH cells are markedly stimulated: hormone synthesis (development of Golgi area and endoplasmic reticulum) and release (reduction of secretory granules) have been observed. This stimulatory serotonergic pathway seemed to be antagonistic to the dopaminergic system that inhibits MSH secretion in the eel. MSH cells have PbH+ granules. They predominate in the pars intermedia. PAS+ cells have smaller and denser granules. MSH cells have two types of granules: the most abundant granules are scattered all over the cell and accumulate in the elongated basal process in contact with the basal lamina and the perivascular channels. The granules are irregular in shape and of a low or medium electron density. They correspond to the PbH+ granules. Their average diameter is 250nm. Other granules are much larger in size (upto 1.25  $\mu\text{m}$ ) and less nume-



rous, and have a fibrillar structure. They probably contain glycoprotein as they are PAS+. The nucleus is situated in the enlarged part of the cell. The poorly developed endoplasmic reticulum is situated in the apical region, distal to the perivascular space. The Golgi apparatus is small. Agranular cells are also present. The cytoplasmic processes infiltrate among MSH cells. They have a few small mitochondria. Olivereau and Chambolle(1979) stated, "Thus, various neurotransmitters appear to be involved in the complex control of MSH secretion in the eel. Among them, dopamine and 5-HT seem to play a major, but antagonistic role. The interaction of other factors like anti- $\alpha$  endorphin-reacting peptides (Follenius and Dubois, 1978 a and b) or of other still unidentified factors remains possible".

#### *Earlier electron microscopic observations in teleosts*

Knowles and Vollrath(1966) studied changes in the pituitary of the migrating European eel during its journey from rivers to the sea. In the Danish eels, no alcian blue-positive material was found in the neurosecretory tracts leading to rostral pars distalis. In the region of the somatotrophic (STH) and gonadotrophic cells in the proximal pars distalis, however, some of the tracts contained a little material. Ultrastructurally a few typeA granules were noted in the tracts leading to the proximal pars distalis. These granules are smaller (c. 1,000Å) than the typeA granules noted in river eels (c. 1,400Å).

The proximal pars distalis contained fibre tracts with many electron-dense typeB granules which are smaller (c. 500Å) than those noted in similar areas (c. 700Å) of river eels. In the same area the terminals of typeB fibres contained plenty of smaller electron-lucent vesicles (synaptic vesicles?).

TypeA vesicles were not found in the tracts leading to the rostral pars distalis and the typeB innervation was same as noted in the river eels.

The granules in the follicle cells of the *rostral pars distalis* measured c. 2,000Å in contrast to the *Prolactin granules* (c. 2800Å) and the TSH granules (c. 1,400Å) of the river eels. Membranebound vesicles and unidentifiable material were noted in the centres of some follicles which possibly correspond to the alcian blue-positive material seen under light microscope. ACTH cells were noted in the rostral pars distalis.

The *proximal pars distalis* is larger than the rostral pars distalis. The STH cells had prominent endoplasmic reticulum. Previously the authors thought that non-STH cells having granules of 1,300Å are possibly FSH cells and those having granules of 1,900Å are possibly LH cells. The authors in 1966 found the following types of GTH cells in the Danish eels.

(a) *Typical FSH cells*: The cell had one vacuole and few slightly electron-dense granules c.1,300Å to c.1,800Å or more, in diameter. The perinuclear space was large and more cytomembrane systems were noted.



(b) "Spaghetii" cells: These cells had densely packed tubules containing moderately electron-dense material. The tubules were surrounded by membrane-bound granules having greater electron density. These granules ranged from c.1,200Å to 1,800Å in diameter. Secretory material within the tubules was surrounded by membranes and thus secretory granules were formed.

(c) Cells having deeply indented nuclei, multiple mitochondria with prominent cytomembrane systems were also noted. Granules in these cells varied from 1,200Å to 1,800Å in diameter. Big empty vesicles filled the greater part of their cytoplasm.

(d) Typical LH cells having electron-dense vesicles c.1,900Å in diameter were noted.

Knowles and Vollrath(1966) concluded that important changes occur in the pars distalis of the pituitary of migrating European silver eels when they enter the sea. A decrease of typeA neurosecretory material in all nerve tracts which penetrated the pars distalis and an increased activity of typeB neurosecretion in the tracts of the proximal pars distalis were found.

There are changes in the gonadotrophic cells of the proximal pars distalis and thyrotrophic and prolactin cells in the rostral pars distalis.

### Teleostei (Some earlier observations)

EM studies of the teleost adenohypophysis have been done by :

Investigators	Types of animal
Follenius and Porto(1960, 1961)	<i>Perca fluviatilis</i> , <i>Poecilia reticulata</i> , <i>P. sphaenops</i> , and <i>Xiphophorus helleri</i>
Weiss(1965)	<i>Xiphophorus maculatus</i> , and other cyprinodonts
Oztan(1966)	<i>Zoarces viviparus</i>
Knowles and Vollrath(1966)	<i>Anguilla anguilla</i> and <i>Conger conger</i>
Vollrath(1967)	<i>Hippocampus kuda</i>

Types of animals	Part of the pituitary	Cell type	EM observations
<i>Perca</i>	Proadenohypophysis	Acidophil	Secretion granules are spherical, membrane-bound, osmiophilic, 120-160nm in diameter.
Cyprinodonts	"	"	Granule-diameter is 200 to 250nm.



<i>Types of animals</i>	<i>Part of the pituitary</i>	<i>Cell type</i>	<i>EM observations</i>
<i>Hippocampus</i>	"	"	90 to 110nm.
<i>Zoarcetes</i>	"	"	Secretion granules are rod-shaped, rounded or forked. They are 400 to 600nm long and 120 to 200nm in width.
<i>Anguilla</i>	"	"	Secretion granules are 280nm in diameter.
<i>Conger</i>	"	"	Secretion granules are elongated & of 350nm in diameter.
<i>Perca</i>	Proadenohypophysis	Cells lining the neurohypophysis	Few granules having size & electron density similar to those in other cells of the rostral pars distalis.
<i>Eels</i>	"	"	Osmiophilic granules are 200 to 250nm in diameter. They are spherical or subovate in <i>Anguilla</i> and sometimes elongate in <i>Conger</i> .
<i>Eels</i>	Proadenohypophysis	Basophils	Large number of spherical, slightly electron-dense, membrane-bound granules of 110 to 140nm in diameter.
<i>Eels</i>	Ventral zone of the mesoadenohypophysis	Basophils	<i>Type I</i> : More or less elongated, electron-dense vesicles of 190nm in diameter. <i>Type II</i> : A few spherical, slightly electron-dense vesicles of 130nm in diameter. The cells have occasional larger secretory granules.
<i>Perca</i> & <i>Cyprinodonts</i>	Mesoadenohypophysis	Basophils	One type only. In <i>Perca</i> no granules. In cyprinodonts the osmiophilic granules were of 100 to 120nm in diameter.



Types of animals	Part of the pituitary	Cell type	EM observations
<i>Zoarces</i>	"	"	Three types. Peripheral type I : -diameter of the secretory granules ranges from 60 to 160nm. Peripheral type-II : 80-240 nm. Antero-central basophilic-globular electron-dense granules of 400nm in diameter.
Cyprinodonts	"	Acidophils	Diameter of electron-dense, membrane-bound granules is 140 to 160nm.
Hippocampus	"	"	210-250nm granules
<i>Perca</i>	Mesoadenohypophysis	Acidophils	250 to 300nm granules
<i>Zoarces</i>	"	"	240 to 320nm "
Eels	"	"	400nm "
<i>Conger</i>	"	"	Granules less electron-dense and more elongate.
Cyprinodonts	Metaadenohypophysis	Cells	Electron-dense granules in cells not noted.
<i>Perca</i>	"	"	Membrane-bound electron-dense granules of 250 to 300nm in diameter. Vesicles with slightly osmophilic homogeneous contents.
<i>Zoarces</i>	"	"	Rounded granules vary in electron-density and diameter is from 240 to 400nm.
	"	Cells corresponding to PAS+ cells of LM.	Electron-dense cytoplasm and granules of 160 to 200 nm in diameter.







## CHAPTER 11

### THE PITUITARY OF DIPNOI AND COELACANTHIFORMES

The description of the hypothalamus of Dipnoans is from Kuhlénbeck(1977). Anterior to the chiasmatic ridge the hypothalamus of *Ceratodus* contains a preoptic nucleus. The nucleus can be divided into a thicker superior and a thinner inferior subdivision. The anterior portion of a nucleus entopeduncularis exists as scattered elements within the basal forebrain bundle. In the postoptic hypothalamus the periventricular cells are differentiated into a dorsal and a ventral subdivision. Near the tuberculum posterius which is a transitional place between the deuterencephalic tegmentum and diencephalic longitudinal zones (thalamus ventralis and hypothalamus), there are two recesses. Recessus mammillaris has been designated to the ventral one (Holmgren and van der Horst, 1925). A saccus vasculosus below it has been mentioned by the same authors. Kuhlénbeck(1977) thinks its presence to be doubtful. The posterior inferior hypothalamus is less developed and corresponds to the Osteichthyan lobi inferiores S. lateralis.

The hypothalamus of *Protopterus* can be subdivided into a rostral preoptic and a caudal postoptic portion. The neuronal elements are periventricularly arranged but a convincing delimitation of the nuclei is not possible (Kuhlénbeck, 1977). The lobi lateralis (sive inferiores) are moderately developed.

Wingstrand(1966) described the pituitary of the lungfish. It is very similar to that of the urodeles. No pars tuberalis can be seen. A hypophysial cleft forms as a schizocoel in the compact anlage of the adenohypophysis. Practically no sella turcica is evident in *Protopterus* which has a flat gland and the pars distalis is located posterior to the neurohypophysis similar to urodeles. *Neoceratodus* has a deep sella turcica and the pituitary extends ventrally from the brain. The pars distalis is situated anterior to the neurohypophysis. In *Lepidosiren* "the relation is intermediate between the other two".

"The anterior end of the pars distalis, which is in contact with the eminentia, consists mainly of large basophils and corresponds closely to the *Zona tuberalis* described in amphibians. This zone is situated between the tongues of the pars tuberalis in amphibians and can therefore not be interpreted as part of the lateral lobes but rather as a homologue of the processus anterior of amniote embryos. Like the processus anterior, this part of the lungfish gland is the main recipient of the blood from the eminentia". No saccus vasculosus is found.

Kerr and van Oordt(1966) found that in *Protopterus dolloi* the hypophysial cavity gives off diverticula into the pars distalis and the cells belonging to the



pars distalis may be found on the pars intermedia side of the cavity. In adult *Protopterus aethiopicus* the hypophysial cleft is partially occluded at places and a mixture of cell types is found belonging to the pars distalis and intermedia. These authors could discover in the larvae of 18 to 26cm of *Protopterus aethiopicus* very short paired protrusions of the anterior tip of the distal lobe which might be homologous with the lateral lobes of the amphibian pituitary from which the pars tuberalis develops but the cells in the protrusions were similar to those of the nearby pars distalis. With the increase in size of the pituitary these rostral protrusions could not be distinguished.

Kerr and van Oordt(1966) noted three types of basophils and two types of acidophils in the distal lobe of *Protopterus aethiopicus*. The following description is after van Oordt(1968). *Basophils type1*: These cells are round and the coarse granules are aniline blue, PAS, AB, and AF positive. The cell boundary is distinct. In young larvae these cells are centrally situated. In adults they spread more rostrally and ventrocaudally.

*Basophils type2*: These cells are found only in adult animals. The fine granules have staining affinities similar to basophils type1 but they are AF positive only on pretreatment with acid permanganate. They are elongated cells and the cell boundary is ill defined. The capillaries are bordered by these cells and they are situated throughout the distal lobe except the rostral tip. No orangeophilic inclusions could be found.

*Basophils type 3*: They are found in the larvae very shortly after the appearance of basophils type 1. These are small rounded cells with fine granules stainable with PAS and with AF following a Lugol treatment. They are AB-negative. The cells are light violet after Herlant's tetrachrome method or Cleveland and Wolfe's method and are concentrated in the rostral part of the lobe where chromophobic cells are also evident.

*Acidophils type 1*: These are oval or elongated cells and irregularly distributed. The fine granules are erythrosine, orange G, and Luxol fast blue-positive. These are developed early in young larvae.

*Acidophils type 2*: They have less pronounced affinity for erythrosine and a faint affinity for PAS and are located in the caudal part of the lobe. In adults they spread laterally.

The pars intermedia is dorsal to the hypophysial cavity with a thin layer of cells in *Lepidosiren* (Kerr,1933) or consisting of irregular lobules. The lobules develop into hollow epithelial tubules interdigitating with protrusions of the pars nervosa in *Protopterus*. This was also noted by Dawson(1940). It resembles the neurointermediate lobe noted in other groups of fishes, but the nervous and the cellular parts are separated by strong sheets of connective tissue where rich supply of small blood vessels can be identified. Majority of the intermedia cells are



less granulated and they are weakly aniline blue, PAS, AB and AF-positive. Some cells are strongly PAS-positive and Luxol fast blue and orange G-positive.

"The neurohypophysis of *Protopterus* is developed at the posterior end of the horizontal post-optic hypothalamus, which bends dorsally and ends with a pair of hollow, primary branches as in amphibians" (Wingstrand, 1968). Accumulation of nsm in the neural lobe is noted and it develops in the wall between the primary branches and from the medial wall of the latter. Interdigitations between the neural lobe tubules and the intermedia tubules are obtained. Capillaries in thin connective tissue membranes are situated in between the neural lobules and the intermedia tubules. "The wall of these tubules consists of an ependymal layer, a fibre layer, and a palisade layer". The median eminence is situated along the anterior margin of the pars distalis. The wall is thin but a typical superficial palisade zone is present having furrows for the lodgment of dense capillary net which communicates with vessels of the pars distalis.

van Oordt(1968) attached different functions to different cell types. Basophils type 1 and type 2 are the sources of TSH and FSH respectively. Eosinophils might secrete STH.

Zambrano and Iturriza(1973) found nerve fibres among the endocrine cells of the pars distalis of *Lepidosiren*. These fibres came from small aminergic neurons situated in the median eminence area and also from other hypothalamic regions. There are type B large granulated vesicles (LGV) in these fibres. These type B fibres end in the pars distalis, pars intermedia and neural lobe close to the pars intermedia. The preoptic nucleus is anterodorsal to the optic chiasma. The perikarya below the ependyma of the median eminence have LGV of 90-100nm in diameter having resemblance to the nucleus lateralis tuberculi of teleosts and infundibular and arcuate nucleus of tetrapods. Lysosomes and secretory granules of 140-190nm in diameter have been noted by the authors in the cells of the preoptic nucleus. Short dendrites from these cells project into the CSF between the ependymal cells. The preopticoneurohypophysial tract arises from these neurons. Two tracts proceed posteroventrally and at the median eminence each tract divides into a dorsal and a ventral part. The axons of the ventral part end around the primary capillary vessels of the median eminence. The blood vessels are branches of hypothalamic arteries. The primary capillaries in the median eminence contact with the palisade layer and have connections with the vessels of the pars distalis. In some cases a second portal system is noted in the neurointermediate lobe by minor connections between the primary capillary bed and the vessels of the neurointermediate lobe but this lobe has an independent blood supply mostly. Different kinds of neurosecretory nerve endings on the pericapillary spaces have been observed and the primary capillaries are fenestrated.

*Type A1* : Irregular electrondense secretory granules are of 140-180nm in diameter.

This type of ending is most commonly noted.

*Type A2* : This is a scarce type. Granules are 120-130nm in diameter.

*Type A3* : Pale granules of 140nm in diameter.



All the endings have clear *synaptic* vesicles and no typeB or typeC endings are evident. In the posterior part of the median eminence TypeA1 fibres end on a thick avascular sheet of connective tissue separating the endocrine cells of the pars distalis. Short portal vessels join the primary capillary bed with the rostral end of the pars distalis.

The dorsal part of the preopticonurohypophysial tract and few fibres of the ventral tract enter the neural lobe.

The plexus intermedius is situated between the neural and intermedia tissues. Zambrano and Iturriza(1972, 1973) found an independent blood supply from the basilar artery. The pars intermedia is practically avascular. The preopticonurohypophysial tract ends on the plexus intermedius. Pars intermedia cells are directly innervated.

Zambrano and Iturriza(1972) studied the neural lobe of the *Lepidosiren* ultrastructurally. The neural lobe is formed by hollow lobules or follicles containing ependymal cells, a fibrous layer and AF-positive outer palisade layer. The ependymal cells have granules of 140-200nm in diameter and have end-feet abutting against the perivascular membrane. Nonsynaptoid contact between the neurosecretory fibres and the ependymal cell membrane is sometimes met with. Gaps exist in between end-feet for the neurosecretory endings. The authors suggested that the neurosecretory material is released into the perivascular space and some nsm is transported through the ependymal cells for release into the infundibular recess. There are four types of nerve fibres in the palisade layer. All of them have *synaptic vesicles*.

*TypeA1* : The electrondense secretory granules are spherical and of 150-180nm in diameter.

*TypeA2* : Irregular dense granules of 130-150 nm in diameter.

*TypeC* : Only clear *synaptic* vesicles.

*TypeB* : Aminergic fibres having large granulated vesicles of 90-100nm in diameter.

#### *The pituitary of the Coelacanthiformes (Latimeria chalumnae)*

Kuhlenbeck(1977) in his book, *Derivatives of the prosencephalon: diencephalon and telencephalon* vol. 5, part I (Publisher : S. Karger) discussed the arrangement of the hypothalamus and the pituitary of the *Crossopterygian Coelacanth Latimeria* in pages 95 and 202-206. *Latimeria* is a highly aberrant form with peculiar distorted pattern of the diencephalon. However, the longitudinal diencephalic zones have a typical vertebrate arrangement. The ventral posterior (postoptic) hypothalamus shows the most striking distortion in the way of being still topologically caudobasal, yet itopographically it has become rostrobasal. The postoptic hypothalamus is situated ventral to the chiasmatic ridge and extends below the telencephalon. The rostrally displaced posterior inferior hypothalamus continues into the neurohypophysial *peduncle hypophysaire* of



Millot and Anthony(1965). This *peduncle* is continuous rostrally with adeno-hypophysial peduncle (*cordon conjonctive-vasculaire*). "The adeno-hypophysis is connected with the palate by a vascularized, partly hollow stalk containing islets of adeno-hypophysial tissue and presumably corresponding to *Rathke's pouch*".

A second, basal *preoptic* but topologically postoptic recess ventral to true preoptic recess and chiasmatic ridge can be seen. The posterior recess of the posterior inferior hypothalamus (*lobi inferiores*) is situated ventral to the tuberculum posterius. It seems doubtful that the saccus vasculosus is somehow connected with the rostral basal hypothalamic floor. The ganglion habenulae is rather caudally placed. "As far as can be inferred from the observations and illustrations published by Millot and Anthony, some parts of the ganglion habenulae show a compact cellular grouping, while thalamus dorsalis, thalamus ventralis, and hypothalamus seemingly display a rather nondescript, diffuse, and essentially periventricular cellular arrangement".

The stria medullaris system, the basal forebrain bundle, and the fasciculus retroflexus can be easily identified. The dorsal portions of the basal forebrain bundle run through the thalamus ventralis instead of the hypothalamus. Others run through the boundary zone of hypothalamus and ventral thalamus. The diencephalic fibre systems also include fasciculus hypo-halamo-peduncularis, fasciculus thalamicus dorsoventralis, fasciculus thalamo-hypo-halamicus caudalis, fasciculus thalamo-hypothalamicus rostralis, fasciculus thalamo-peduncularis and fasciculus thalamo-tegmentalis.

The commissural decussating systems comprise of commissura anterior, commissura habenulae, commissura posterior, optic chiasma and postoptic commissure. Similarly the crossing fibres seem to be present near the tuberculum posterius at the basal diencephalomesencephalic boundary zone.

The description of the pituitary is from Millot and Anthony(1955,1959,1965) and Lagios(1972). A part of the adeno-hypophysis is situated under the brain in the posterior part of the cranium having contact with the digitations of the neurohypophysis. The neural lobe is the anterior process of the infundibulum and its interdigitations with the pars intermedia are as noted in the fish (Lagios, 1972). The main part of the pars distalis is found on a depression situated dorsally over the neurointermediate lobe. This part of the pars distalis is called the *cerebral pars distalis* (Holmes and Ball, 1974). From the cerebral pars distalis a long cylinder of fibrous tissue projects anteroinferiorly for 10cm and ends at the roof of the buccal cavity. Lagios(1972) found a large mass of pars distalis cells at the buccal end of the projection. This is called *buccal pars distalis* (Holmes and Ball,1974). Within the fibrous cylinder well vascularized islets of pars distalis cells can be found. Millot and Anthony(1965) thought that the fibrous cylinder is the persistent and elongated stalk of Rathke's pouch. Follicles of acidophilic cells filled with colloid is found in buccal pars distalis. There are



also well vascularized cords of cells. Lagios(1972) found central cystic spaces which may be the residual hypophysial cavity. The anterior part of the cerebral pars distalis has acidophils and Lagios could not identify the cell types of the posterior part as the fixation was not good. There was a small saccus vasculosus evaginating from the posterior infundibular wall.

**Blood supply** (Lagios,1972): The internal carotid arteries enter the cranial cavity in close approximation to the buccal pars distalis converging on the fibrous cylinder. This part of the pituitary may receive some fine branches. The internal carotid arteries being ensheathed in the tubular fibrous tissue proceed towards the optic chiasma and ventral to it they bifurcate and a group of convoluted arterioles arise from them. Primary capillaries of the median eminence are fed by these vessels. These are perpendicular glomeruloid capillary complexes and each of them is separately ensheathed in fibrous tissues at the rostral aspect of the infundibulum. At the median eminence some neurosecretory fibres end on the primary capillaries. These capillaries become continuous with the dilated vertical venous channels which enter into the cerebral pars distalis and proceed into the fibrous cylinder and reach the buccal pars distalis and supply the same (Holmes and Ball, 1974).

Lagios and Stasko-Concannon(1979) described the presumptive interrenal tissue (adrenocortical homology) of the coelacanth *Latimeria chalumnae*. Partially encapsulated bright yellow corpuscles were situated in the walls of the posterior caval veins and their major tributaries. Large vacuolated cells formed the corpuscles. The cells contained lipid and cholesterol. Ultrastructural examination of the cells showed plenty of liposomes which may indicate inactiveness.

A provisional study on the identity of corticosteroids of the coelacanth *Latimeria chalumnae* Smith was conducted by Truscott(1980). The presumptive adrenocortical cells situated in the coelacanth kidney (frozen tissue) was analysed by double isotope derivative assay (DIDA) for adrenocorticosteroids. Evidence was obtained for the presence of 11-deoxycorticosterone, cortisol, and corticosterone. 11-deoxycortisol or cortisone could not be detected. When this tissue was incubated with exogenous radioactive steroid precursors, some transformation products could be identified which indicated the presence of active enzyme systems including 5-ene-3 $\beta$ -hydroxysteroid dehydrogenase, 5-ene-4-ene-isomerase, 20 $\alpha$ -hydroxysteroid dehydrogenase, 11 $\beta$ -hydroxysteroid dehydrogenase, and pregnene-5 $\alpha$ - and 5 $\beta$ -hydrogenases.

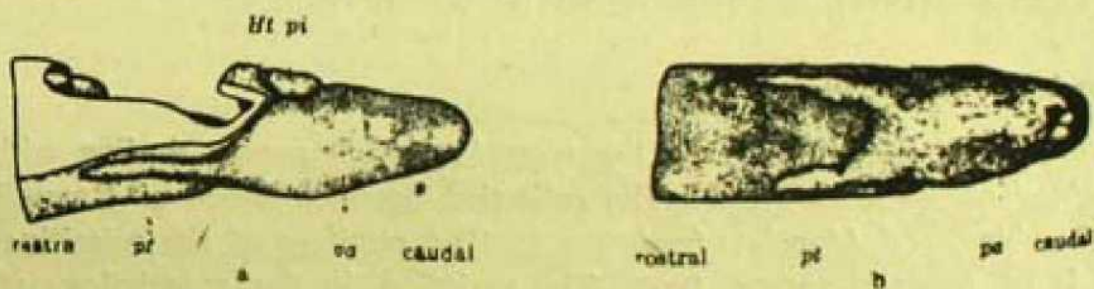
Hayashida(1977) conducted immunochemical and biological studies with growth hormone in a pituitary extract of the coelacanth, *Latimeria chalumnae* Smith. The substance in the coelacanth pituitary extract "shows a low, but significant degree of relatedness to GHs of tetrapods and to a substance immunochemically related to GHs in pituitary extracts of other existing primitive fishes including the lungfish, shark and sturgeon. Bioassay of the pituitary extract revealed that it was capable of a low, but significant degree of stimulation of growth promotion in a mammal on the basis of the rat tibia assay."



## CHAPTER 12

### THE PITUITARY GLAND IN AMPHIBIA

The pituitary of the lungfish has many features common to that of the amphibians (Wingstrand, 1956). Wingstrand (1966) described the development in details. In both these animals Rathke's pouch develops as a compact epithelial bud. It remains compact in Anura, and Urodela. A schizocoelic lumen is formed in the compact epithelial bud which disappears during further development in Gymnophiona. The schizocoelic lumen remains as a hypophysial cleft in the adult gland of Dipnoi. In Urodeles, Anurans and Protopterus the situation of the adenohypophysis is either posterior or ventrocaudal to the neurohypophysis. The posterior pole of the gland is formed by the pars distalis. The lungfish has no pars tuberalis. In the amphibians the pars tuberalis develops as a pair of tongue shaped outgrowths which project anteriorly from the anlage of the adenohypophysis. These outgrowths may remain in contact with the pars distalis in Urodela and Gymnophiona. They are isolated as two patches of epithelial tissue on the ventral surface of the tuber as in Anura (figs. 12.0a, 12.0b, and 12.0c).



Figs. 12.0a and 12.0b. Waxplate reconstruction of the hypophysis and the adjoining part of the brain of an adult *Ambystoma jeffersoni*.

Ht = Derivative of the brain; pa = Pars anterior; pi = Pars intermedia; pt = Pars tuberalis;

(a) View from the side

(b) View from ventral aspect.

X 1:28. After Atwell (1921, Figs. 8 and 10).

From B. Romeis. Courtesy of Springer-Verlag.

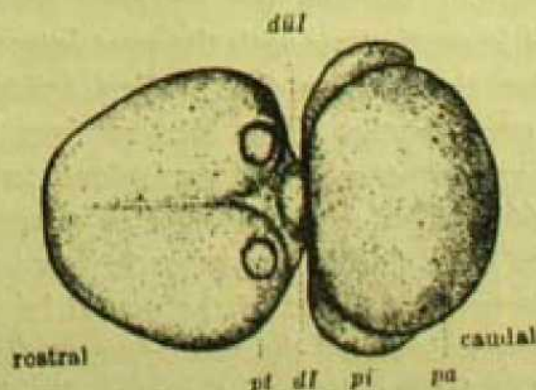


Fig. 12.0c. Ventral view of infundibulum and the hypophysis of *Rana catesbeiana*; pa = Pars anterior, pi = Pars intermedia; pt = Pars tuberalis; dl = Thinner part of infundibular floor; dul = Thinner part of infundibular floor.

X 1:10. After Atwell (1919, Fig. 15).

From B. Romeis. Courtesy of Springer-Verlag.



The *Gymnophiona* has a long infundibular stem. Except in *Gymnophiona* the neurohypophysis is characterized by wide and backwardly directed saccus infundibuli which ends in a pair of diverticula and this corresponds to the primary branches of amniote neural lobes. The development of the neural lobe is from the posterior wall of the saccus infundibuli between these primary branches and from the posteromedial wall of these branches. The neural lobe is small and its separation from the median eminence is indistinct. The median eminence is often poorly differentiated and it is situated in the wall immediately ventral to the neural lobe.

The pars intermedia is dorsal or dorsocaudal to the pars distalis. It is continuous with the pars distalis in urodeles. In anurans the pars intermedia is connected at its caudoventral rim with the dorsocaudal surface of the pars distalis. The rostral surface of the pars intermedia is attached to the caudoventral surface of the pars nervosa of the neurohypophysis.

Excellent reviews on the comparative morphology of the pituitary are those of Green(1951), Herlant(1954), Green and Maxwell(1959), Dodd(1963), Dodd and Kerr(1963), Wingstrand(1966), van Dongen *et al.*(1966), van Oordt(1968), Holmes and Ball(1974), and Fontaine and Olivereau(1975).

#### *The pituitary of the anura :*

Wingstrand(1966) mentioned the most important papers dealing with the morphology of the adult gland. His collection contains series of *Rana*, *Bufo*, *Hyla*, *Bombina*, and *Liopelma hochstetteri*. The pituitary of the primitive *Liopelma* is of common anuran type. The variation in the morphology of the pituitary in anurans is very small.

#### *The adenohypophysis*

The adenohypophysis is situated posterior or ventrocaudal to the neurohypophysis. The pars distalis of anurans is somewhat flattened dorso-ventrally and is often broader than long forming the posterior pole of the pituitary and is exceptionally broad. It is continuous with the pars intermedia along its antero-dorsal margin. Connective tissue joins the median eminence with the antero-ventral extremity of the pars distalis. This area receives blood from the portal vessels of the eminentia and appears to be equivalent to the mammalian zona tuberalis (Wingstrand,1966). The *pars tuberalis* is a small cell plate, closely attached to the surface on each side of the tuber in front of the pituitary. The *intermedia* covers the postero-ventral part of the neural lobe. The transverse bar formed by these two structures is called the *neuro-intermediate lobe*. It is broader than the pars distalis. The intermedia and the pars distalis is not separated by a hypophysial cleft.

The *neurohypophysis* of anurans is better developed than in urodeles and lungfishes and wide capillaries supply the neural lobe. These capillaries partly



pass through the tissue and partly run in furrows on the surface. The separate blood supply of the neural lobe is shared only by the intermedia. The neural lobe contains plenty of AF+ neurosecretory material. The common blood supply to the neural lobe and the pars intermedia is through the plexus intermedius situated between these two structures (Wingstrand, 1966). The *median eminence* is antero-ventral to the neural lobe. It is very near the antero-ventral end of the pars distalis having contact with the same. On the ventricular side, the wall of the median eminence is lined with ependyma and the fibre bundles of the hypothalamo-neurohypophysial tract are below the ependymal lining. The thick external zone of the median eminence contains AF+neurosecretory material, although significantly less than the neural lobe. Processes of plenty of glial cells (pituicytes) form palisade zones around the numerous capillaries which are partly embedded in the substance. These primary portal capillaries are drained by portal vessels which pass to the pars distalis. A thin undifferentiated wall separates the eminentia from the neural lobe. This can be regarded as a ventral wall of an infundibular stem forming part of the roof of a large recess between pars distalis and pars intermedia (Holmes and Ball, 1974).

#### *The pituitary of urodela :*

Wingstrand (1966) reviewed the urodelan pituitary and his collection includes series of Triton, Ambystoma and Salamandra. The pars distalis of the *adenohypophysis* is similarly situated as in anurans but it is more elongated and contains large glandular cells. The *zona tuberalis* is formed at the antero-ventral end of the gland where the portal vessels enter. In Salamandra the *intermedia* is much better developed laterally than in the median parts. In all the forms the *pars tuberalis* is present as a pair of epithelial strings or tongues. They grow out from the antero-ventral end of the pars distalis on each side and are attached to the surface of the tuber.

The differentiation of the *neurohypophysis* is same as in anurans except the median eminence which is always less well developed than that of anurans. The neural lobe is thick, compact and vascular. It contains AF+neurosecretory substance and is lobulated in the majority of forms, but the neural lobe of Amphiuma and Necturus sends hollow lobules into the intermedia as is found in Dipnoi (lungfishes). The *median eminence* is very poorly developed in Necturus, Cryptobranchus and Megalobatrachus. "The wall of the hypothalamus which is in contact with the pars distalis antero-ventrally of the neural lobe is covered by a capillary net, which is continuous with and drained into the vascular bed of the pars distalis, but the connecting vessels have not fused to a few, prominent portal vessels, and the wall has not thickened to form a distinct median eminence, although the glia fibres below the vessels may show a palisade-like arrangement. In other forms, such as Triton, the wall under the primary plexus of the portal system is thicker and the vessels are partly buried in the proliferated palisade



Zone. This eminentia is, however, confluent with the neural lobe in median sections, and the limit between the organs is best seen in preparations stained for neurosecretory substance, which is stored mainly in the neural lobe" (Wingstrand, 1966).

*The pituitary of the Gymnophiona (Apoda) :*

Most striking difference exists in the apodan pituitary when compared to that of other amphibians (Wingstrand, 1966). The entire gland is very flat. The *pars distalis* is anterior to the neural lobe and the posterior end of the pituitary is formed by the neural lobe. The intermedia is developed along the anterior part of the neural lobe, where it is in contact with the adenohypophysis. As in urodeles, a pair of tongue-like lateral lobes develops from the anterior end of the adenohypophysial anlage. These structures are incompletely detached from the main gland and therefore the position is intermediate between those of Urodela and Anura. In early stages of embryonic development a small lumen can be seen in Rathke's pouch but it disappears later (from Wingstrand, 1966).

The infundibular stem is very long and thin. "It passes dorsally to the *pars distalis* in a furrow to the caudally situated neural lobe, which is a fairly compact structure. In spite of its narrowness, the stem contains a minute recess from the ventricle, and this recess widens into a small cavity in the basal part of the neural lobe. There is a portal system as in typical tetrapods, and the primary plexus is situated on a modified brain surface near the front end of the *pars distalis*. This modified area deserves the name *eminentia mediana*" (Wingstrand, 1966).

*Blood supply of the pituitary*

The hypophysio-portal vessels are like those of tetrapods but the supply to the neurointermedia is almost entirely portal. Rodriguez and Piezzi (1967) studied the vascular supply in *Bufo* in details. Capillaries of the primary portal plexus in the median eminence are fed by branches from the hypophysial artery. Portal veins or capillaries in urodeles are formed which supply the secondary plexus in the *pars distalis*. Direct arterial supply to the plexus intermedius through large capillaries is from the hypophysial artery. Some primary capillaries in the median eminence pass along the infundibular floor to end in the plexus intermedius.

The second portal system is well-developed and is called the *encephalo-neurohypophysial portal system*. Primary plexus of this system is situated in the mesencephalon and the hypothalamus near the preoptic nucleus. The plexus is supplied by the basilar and retroinfundibular arteries. In the roof of the infundibular recess, downflow of blood is collected in one or two very large vessels which proceed to the neural lobe and form intrinsic capillaries of the neural lobe



and supply the plexus intermedius. The plexus intermedius gets blood supply from the following sources :

- (a) the median eminence
- (b) the hypophysial artery
- (c) the encephalo-neurohypophysial-portal system (maximum quantity of blood supplied).

Small capillaries at the periphery of pars intermedia carry blood from the plexus intermedius and some vessels pass to the dorsocaudal part of the pars distalis. The amphibian neural lobe is penetrated by numerous capillaries (Rodriguez,1971). Large capillaries form a type of perforated diaphragm separating the neural lobe from the pars intermedia. This resembles the vascular septum of the lamprey and lizard neural lobes. The *mammalian* neural lobe is penetrated by a large number of blood vessels. These vessels appear to be branches of the arteries supplying the posterior lobe and they do not belong to the secondary plexus of a portal system.

Neural lobe blood is carried through the hypophysial vein and thus the neurohypophysial octapeptides pass to the systemic circulation and it is important to *Bufo* for water conservation problems (Jorgensen *et al.*,1969). From plexus intermedius separate veins proceed to the hypophysial vein. Pars distalis blood passes to the hypophysial veins through the posterior collecting veins directly or through the plexus of the endolymphatic sacs.

Pars intermedia receives blood from neural lobe and also from the median eminence, hypothalamus, mesencephalon and general circulation. Pars distalis receives blood supply mainly from the median eminence and partly from the vessels of the neurointermedia (posterior supply-meagre). The A2 cells are most likely to be exposed to blood from the plexus intermedius (Holmes and Ball,1974). For the transport of MSH long distances are required from the endocrine cells to the capillaries as the pars intermedia is less vascular.

### *The hypothalamus*

Diffuse parvocellular nuclei corresponding to those in the hypophysiogenic area of the higher vertebrates have been noted in the *Bufo melanostictus* as evidenced by pituitary grafting experiments (Roy,1969-71). This has also been found by Jorgensen (1968).

The *preoptic nuclei* are situated in the anterior hypothalamus and in the *Bufo* they can be divided into two groups: one dorsal and the other ventral. The dorsal group is situated high up in the walls of the third ventricle. From these cells AF-positive and PAS-negative fibres end in the median eminence and they are concerned with spermiation. They are type A fibres destined for the median eminence (Rodriguez,1966; Rodriguez, La Pointe and Dellman,1971).



The ventral group lies in the wall of the preoptic recess and the preopticoneurohypophysial tract starts from it. These typeA fibres are AF-positive, PAS-positive and are engaged in water balance of the animal.

*The infundibular nucleus* is situated in the ventral hypothalamus giving origin to typeB fibres which end in the median eminence. The cells are aminergic and may be homologous with the reptilian and avian infundibular nucleus and the arcuate nucleus of the mammals (Zambrano and De Robertis, 1968). Dierickx (1966, 1967) found the gonadotrophic centre to be located in the basal hypothalamus, the pars ventralis tuberis. Aldehyde fuchsin-negative parvocellular nuclei from this part send typeB fibres to the median eminence. Infundibulum of the brain of *Triturus cristatus* was studied with scanning electron microscopy by Fasolo *et al.* (1979).

*Posterior aminergic nuclei* are localized in the posterior hypothalamus. Light and electron microscopic histochemistry indicates that the tracts are monoaminergic (Rodriguez, 1972). The typeB fibres take their origin in a pair of long curved nuclear groups having AF-negative cells, in larval *Xenopus* and adult *R. temporaria*, *Triturus cristatus* and *Bufo bufo* (Peute and Goos, 1970; Fasolo and Franzoni, 1971; Rodriguez, 1972). These neurons, preoptic nuclei and infundibular nuclei have ventricular processes protruding into the third ventricle. The fibres from the posterior aminergic nuclei run in a tract near the median eminence and enter into the pars intermedia. These fibres along with the typeA preopticoneurohypophysial fibres course through the internal region of the median eminence and divide opposite to the pars intermedia. One part ends on the wall of the capillaries which connect the primary portal plexus with the plexus intermedius. The other part pierces the neurointermedia septum and some typeB fibres end on the capillaries and the majority pass into the pars intermedia (Doerr-Schott and Follenius, 1970; Rodriguez, 1972). Peute and Goos (1970) thought that these nuclei in the posterior hypothalamus elaborate an MSH-inhibiting monoamine.

In amphibians (frog and newt) the posterior hypothalamus is related to the median eminence (Fasolo and Franzoni, 1978). Distinct tracts arising from discrete areas in the periventricular gray matter and directed towards the neurohypophysis have been observed after application of HRP in the frog.

Fluorescent reactions in the preoptic area, ventral hypothalamus, median eminence and neural lobe of *Triturus helveticus*, *Pleurodeles waltlii*, *Rana esculenta*, and *Bufo bufo* were noted by Gabrion *et al.* (1978). These reactions denoted vasotocin producing system immunocytochemically. Only the preoptic area contained the immunofluorescent cell bodies. Hypophysectomy greatly increased the fluorescence in *Triturus*. Immunofluorescent axons formed a dense network in the ventromedial hypothalamus behind the optic chiasma. The bundle of fluorescent fibres proceeded towards the ventral surface of the diencephalon and the rostral median eminence near the rostral adenohypophysis containing corticotrophic cells. Immunoreactive axons and Herring bodies were situated in



the inner zone of the caudal median eminence. In *Triturus* and *Rana* a few immunoreactive axons proceeded anteriorly towards the posterior telencephalon.

Doerr-Schott and Dubois(1978) could localise different peptidergic substances in the brain of amphibians and reptiles immunohistochemically. The authors summarized immunohistochemical findings with LHRH-, SRIF-, alpha- and beta-endorphin and met-enkephalin antisera in amphibians and reptiles, interpreted in the light of classical specificity checks.

**Study of GnRH system :** In amphibians, a septo-preoptico-infundibular system could be revealed immunohistochemically by the use of LHRH antiserum. In *Xenopus laevis*, small immunoreactive cells were found in a small telencephalic area, near the lateral septal nuclei. In *Rana pipiens* and *Rana catesbeiana* GnRH cells could be found in medial septal nucleus, lateral septal nucleus, diagonal band of Broca, and bed nuclei of the anterior hippocampal commissures. Immunoreactive cells could not be found in the telencephalon of control *Rana esculenta*, *Rana temporaria* and *Bufo vulgaris*. Immunopositive endings could however, be found in the median eminence. This discrepancy can be explained by insufficient content of GnRH in the perikarya. In hypophysectomized *Bufo vulgaris* telencephalic fluorescent GnRH cells were noted. In the anterior preoptic area of *Xenopus laevis* and *Rana esculenta*, another group of GnRH cells has been observed by the authors. The third group of immunoreactive cells is found to be situated along the ventral edge of the infundibulum in *Xenopus laevis* and *Triturus marmoratus*. The septoinfundibular pathway exists in *Xenopus laevis*, *Rana pipiens* and *Rana catesbeiana*.

**SRIF-like system :** It is present in the median eminence of the newt, in the preoptic nucleus and hypothalamo-hypophysial tracts of *Bufo* and *Xenopus*. Immunoreactive perikarya are also present in the posterior hypothalamus in hypophysectomized *Alytes obstetricans* treated with STH.

LHRH and SRIF + fibres have been noted mainly in the external zone of the median eminence in *Xenopus laevis*. The granules containing LHRH are 890 Å in diameter. SRIF + granules are 840 Å in diameter. These two types of granules are situated in two different types of fibres.

**Alpha-and beta-endorphin + neurons** have been identified in some cells of the nucleus preopticus, in a few isolated cells and in plenty of fibres situated in the pars ventralis of the tuber cinereum of *Rana temporaria*. The immunopositive chains in the infundibular floor are perpendicular to the hypothalamo-hypophysial tracts. These chains end over the capillaries. Plenty of immunopositive nerve endings could be found near the pars tuberalis. Such endings were not present in the external zone of the median eminence.

In *Rana temporaria* met-enkephaline + neurons have been noted in the paraventricular organ. Immunopositive axons of unknown origin were found to terminate near the nucleus preopticus.



The authors concluded, "Morphinomimetic peptides affect the regulation of neurohormonal systems which transmit neurohormonal substances towards the portal hypophyseal system, along the hypothalamo-hypophyseal pathway".

### *The paraventricular organ(PVO)*

Vigh(1969) described the structure and function of the paraventricular organ (PVO). This is an ependymal organ situated at both sides of the third ventricle. The monoamines as detected by fluorescence method could be demonstrated in a distinct neuron group called the *nucleus organi paraventricularis* belonging to the organ. The Hungarian investigators in 1966 could demonstrate that the monoamines are not localized in the special ependyma. The free ventricular nerve endings take their origin from the neurons of the nucleus mentioned above. The monoamine containing nucleus forms nerve terminals in the cerebrospinal fluid. It is present from fishes up to birds.

In the posterior recess of the hypothalamus of fishes there is a PVO-like area. Monoaminergic fibre-bundles originate from it. Free nerve endings could also be found at different parts of the brain ventricles. The Hungarian authors detected a PVO-like area in the preoptic recess of amphibia, which they called as *preoptic recess organ*. All these areas are characterized by monoamine-contracting neurons and free ventricular terminals. These morphologically analogous systems are called as *liquor-contacting neuronal systems*. These systems are interconnected with fibres.

### *Functions of the paraventricular organ :*

(1) PVO has a secretory ependyma. In some species Gomori-positive material has been found in the ependymal cells of the organ. The amount of paraventricular *ependymosecretion* varies with the age of the animal. Thus it may be connected with maturity, though it may not be the main function of the organ.

(2) Monoamines may be secreted into the cerebrospinal fluid (*neurosecretory phenomenon*).

(3) The PVO and other liquor-contacting neuronal areas act as a *receptor of the ventricular wall*.

(4) The organ may act as a *chemoreceptor of the cerebrospinal fluid*. The authors studied the fibre connections of the PVO in amphibia. They found "a connection with the monoaminergic area of the preoptic recess, with the neurosecretory nuclei, with the epithalamus including the subcommissural organ, with the monoaminergic nuclei of the mes- and rhombencephalon, and with the median eminence". The authors supposed that "the paraventricular organ informs these mentioned territories about the state of the cerebrospinal fluid, thereby assuring its homeostasis."



Vigh-Teichmann *et al.* (1969) studied the phylogeny and ontogeny of the paraventricular organ. Their study included fishes, amphibians, reptilia, birds and mammals. Human brains were also studied. In the *cartilaginous fish*, *Raja clavata*, PVO is represented by well developed lateral recess of the third ventricle. Similar feature has been noted in the systems of the posterior recesses. In *Scyliorhinus caniculus* a PVO has been noted. The PVO in *bony fishes* is well developed and consists of two divisions differing structurally and in monoamine content. The neurons of the PVO form two layers: a proximal or hypendymal layer contains smaller cells. The distal layer contains larger neurons. Strongly fluorescent fibre bundles take their origin from the PVO and pass into different diencephalic parts.

In *amphibia* the PVO extends into the lateral recess of the infundibular lobe. In urodela another PVO-like area, perhaps analogous to the system of the fishes' posterior recesses is situated in the upper part of the infundibular lobe. The nucleus of the PVO cannot be divided into two parts by a fibrous zone. Fluorescent fibre bundles leave the PVO and the scattered and partly crossing fluorescent bundles proceed towards the following areas: to a monoamine-containing nucleus located in the most caudal part of the diencephalon, and in the mes- and rhombencephalon; to the epithalamus including the subcommissural organ; to the preoptic recess and the median eminence.

The *reptilian* PVO is more differentiated than that of *amphibia*. It is divided into a proximal and a distal part by a characteristic fibrous synaptic layer.

The nucleus of the *avian* PVO is composed of a proximal, a distal and a hypendymal part. They are separated from one another by fibrous synaptic zones. The PVO is connected with the subependymal monoaminergic fibrous zones and the zones on the outer surface of the hypothalamus, with the paraventricular nucleus and the monoaminergic fibres of the preoptic recess.

The wavy paraventricular ependyma of *mammals* may not be considered to be the homologue of the PVO of lower vertebrates.

The authors could establish that, "the PVO of all animals (fishes, *amphibia* and birds) show a well developed structure and differentiation already in early embryological stages. Shortly after the appearance of the PVO's primordium, monoamines can be demonstrated histochemically, suggesting the start of functioning. Therefore, the PVO may play a role in embryonic physiology, too."

Vigh-Teichmann (1969) described hydrencephalocriny of neurosecretory material in *amphibia*: The preoptic nucleus of the frog, *Rana esculenta* was stained with chrome alum galloxyanine method of Bock (1966)). The authors could distinguish two groups of neurosecretory cells. Neurons were situated hypendymally or intraependymally. The other neuronal group was situated further away from the ependyma. The dendrites from both the groups pass between the ependymal cells and protrude into the third ventricle where they form bulb-like endings. Neurosecretory granules have been noted in the nerve cells, the ventricular dendrites and in the ventricular endings.



### *The organon recessus preopticus(ORP)*

It is situated in the rostral part of the preoptic recess where similar neurons form bulb-like ventricular endings. These nerve cells are smaller than the cells of the preoptic nucleus. Gomori + neurosecretory material is not found in these nerve cells. Plenty of monoamines can be demonstrated.

The ORP extends from the terminal lamina to the anterior end of the preoptic nucleus. Monoamine-containing neurons could be observed by them on the whole surface of the preoptic recess and around the anterior commissure, near the foramen of Monro. The ORP is formed by two neuronal groups: *hypendymal* and *diffuse*. The hypendymal cells are *bipolar* and situated within or adjacent to the special stratified ependyma. The nerve cells of the diffuse variety are *multipolar* and situated further away from the ventricular surface. The ventricular processes of both these groups project into the third ventricle and possess bulb-like endings. Monoamines have been noted in the cell bodies, processes and ventricular endings. Dense-core vesicles were noted by the investigators in the perikarya, processes and ventricular endings of both the neuronal groups of the ORP. A well developed organ was found *only* in urodela and anura. The monoamines appear much later in the ORP than in the paraventricular organ. PVO starts functioning earlier than ORP. Monoamine oxidase and AchE were present in good amount in the ORP. G-6-P-DH, LDH, SDH, Glut-DH, NADH-cytochrome-c-reductase, acid and alkaline phosphatases, various nonspecific esterase, BchE and leucine aminopeptidase were moderately active or negative. Green fluorescence was found in the ORP which differs from that noted in the paraventricular organ and the mes-and rhombencephalic nucleus in amphibia. Pharmacological experiments suggested the presence of a primary catecholamine in the ORP.

Crossed fluorescent fibres are situated above and below the ORP. Crossed and uncrossed fibres proceed in two directions from the organ. One bundle runs towards the ventral part of the telencephalon. The other bundle runs into the caudal part of the diencephalon. The diencephalic bundle is in connection with the preoptic nucleus, the paraventricular organ, the monoamine-containing nucleus of the mes-and rhombencephalon, the epithalamus and probably with the fluorescent area of the median eminence.

ORP has a receptor function for the cerebrospinal fluid. The receptor gives special informations about the composition of the cerebrospinal fluid to the different parts of the central nervous system. A secretory function (hydrencephalocriny of monoamines) can also be attributed to the ORP.

### *Median eminence*

Rodriguez(1972) discussed the comparative and functional morphology of the median eminence. The main tissue elements are ependymal and glial cells, nerve fibres and blood vessels. The *internal or neural region* consists of ependymal lining, nerve tracts proceeding towards the neural lobe and long sub-



ependymal loops of the primary plexus of hypothalamo-adenohypophysial portal system. The *external or neurohaemal region* consists mainly of short loops of the portal system, different types of nerve endings, ependymal processes and glial cells. The bird median eminence can be divided into an *anterior* and a *posterior* part according to the affinity for AF. The *rostral region of the median eminence* in mammals and man was considered by Rodriguez as a third region of the mammalian median eminence.

Type B AF-positive fibres end in the median eminence. No Cajal-positive fibres could be observed to end in the external region of the median eminence. Silver chromate method of Golgi showed a rich plexus of nerve fibres ending in the external zone of the median eminence.

Different types of nerve endings depending on the size and structure of the secretory vesicles exist in the external median eminence and there are considerable variations amongst different species (Kobayashi *et al.*, 1970). Rodriguez (1969) described six types of fibres in the toad median eminence. Budtz (1970) and Doerr-Schott (1970) described five types of terminals in the median eminence of the toad *Bufo bufo* and in the frog *Rana temporaria*. Rodriguez (1972) said, "most, or perhaps all, the terminals of the median eminence contain both, a monoamine, and a releasing or an inhibiting factor".

*Short capillary loops* in the median eminence are situated in the external region. They are surrounded by a PAS-positive fibrillar formation which is arranged in a circular and radiating pattern. From electron microscopic observations it could be understood that these formations are long processes of the perivascular basement membrane (fig. 12.1). There are perivascular spaces in some short loops while in others a single basement membrane separates the endothelium from the surrounding nervous tissue. Nerve terminals, glial cells and ependymal processes come in contact with the short loops and the processes of their perivascular basement membrane. Only a few connective tissue cells (fibroblasts, mast and plasma cells) are sometimes noted in the space surrounding the short loops.

In the amphibians *long capillary loops* with ascending and descending branches have been noted by Rodriguez (1969). These are situated in the external median eminence and the subependymal part of the loop usually lies parallel to the ependymal lining. The descending branch of the loop is wider than the ascending one. The perivascular space surrounds the long loops. The external perivascular basement membrane surrounding the subependymal portion generally has numerous and short finger-like projections which intermingle with similar formations of the ependymal endings (Rodriguez, 1972). Nerve terminals, glial cells and ependymal processes come in contact with the ascending and descending branches of the long capillary loops. Basal processes of the ependymal cells and subependymal glial cells form a *cuff* which surrounds the subependymal part of the long loop (fig. 12.2). Perivascular connective tissue cells are rarely found.



Two main types of the ependymal cells could be detected in the median eminence of the toad. One is a short cell without a basal process and the second type is tall with a basal process. The basal processes of the second type of

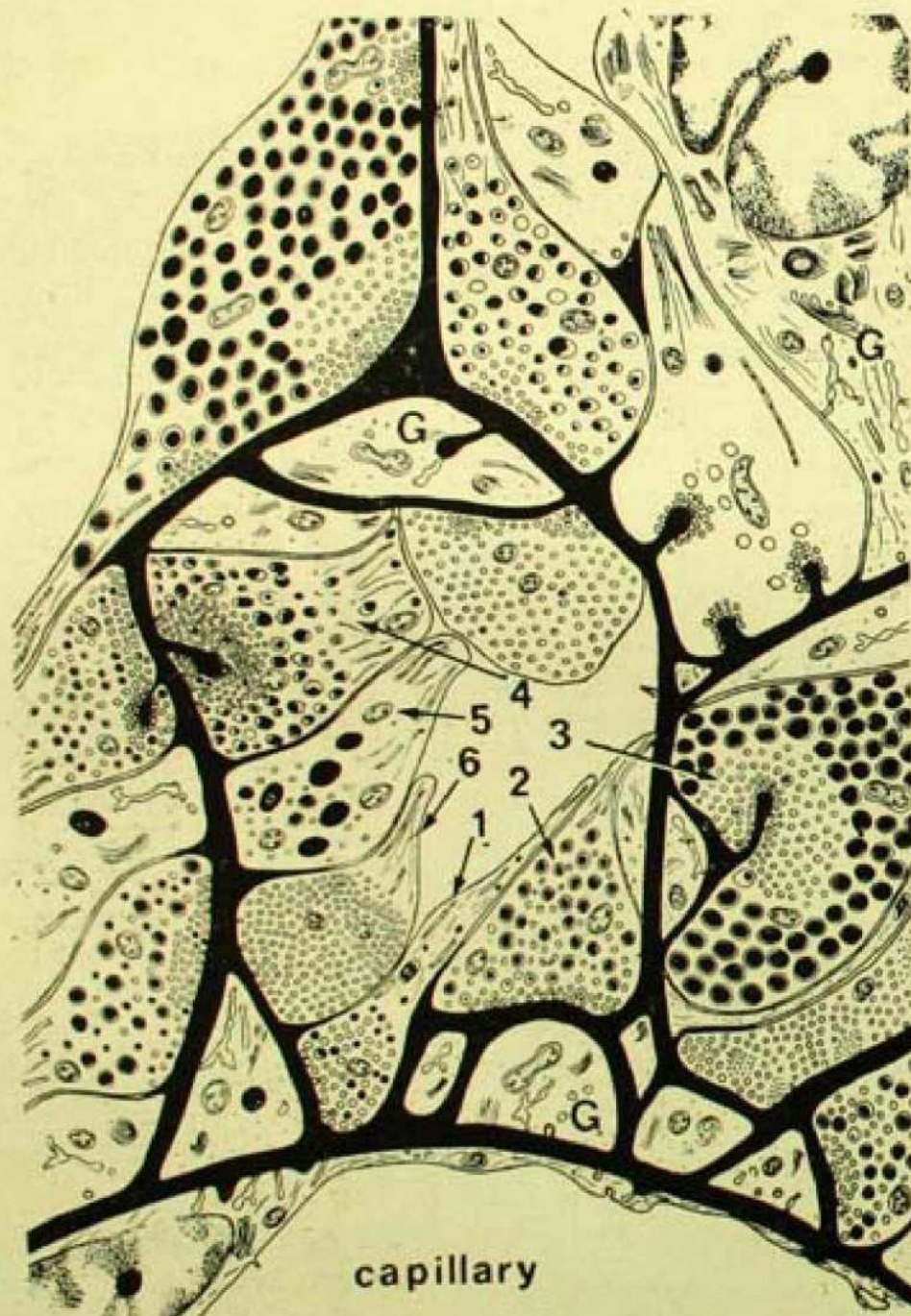


Fig. 12.1 Semi-schematic representation of the interrelationships between a short loop of the toad median eminence and the surrounding nerve elements. 1, 2, 3, 4, 5 and 6 are different types of nerve terminals. The perivascular basement membrane and its processes are shown in black. (G), glial cells—From Rodriguez (1972). Courtesy of S. Karger AG, Basel.

ependymal cells end either on the membrane situated at the external surface of the brain or they end on the perivascular basement membrane of the short and long capillary loops.



The perikaryon of the short and tall ependymal cells is almost completely filled with filaments, tubular and vesicular formations and a few mitochondria. Microvilli are present and few cilia project into the third ventricle. The cells are bound together by tight junctions and desmosomes.

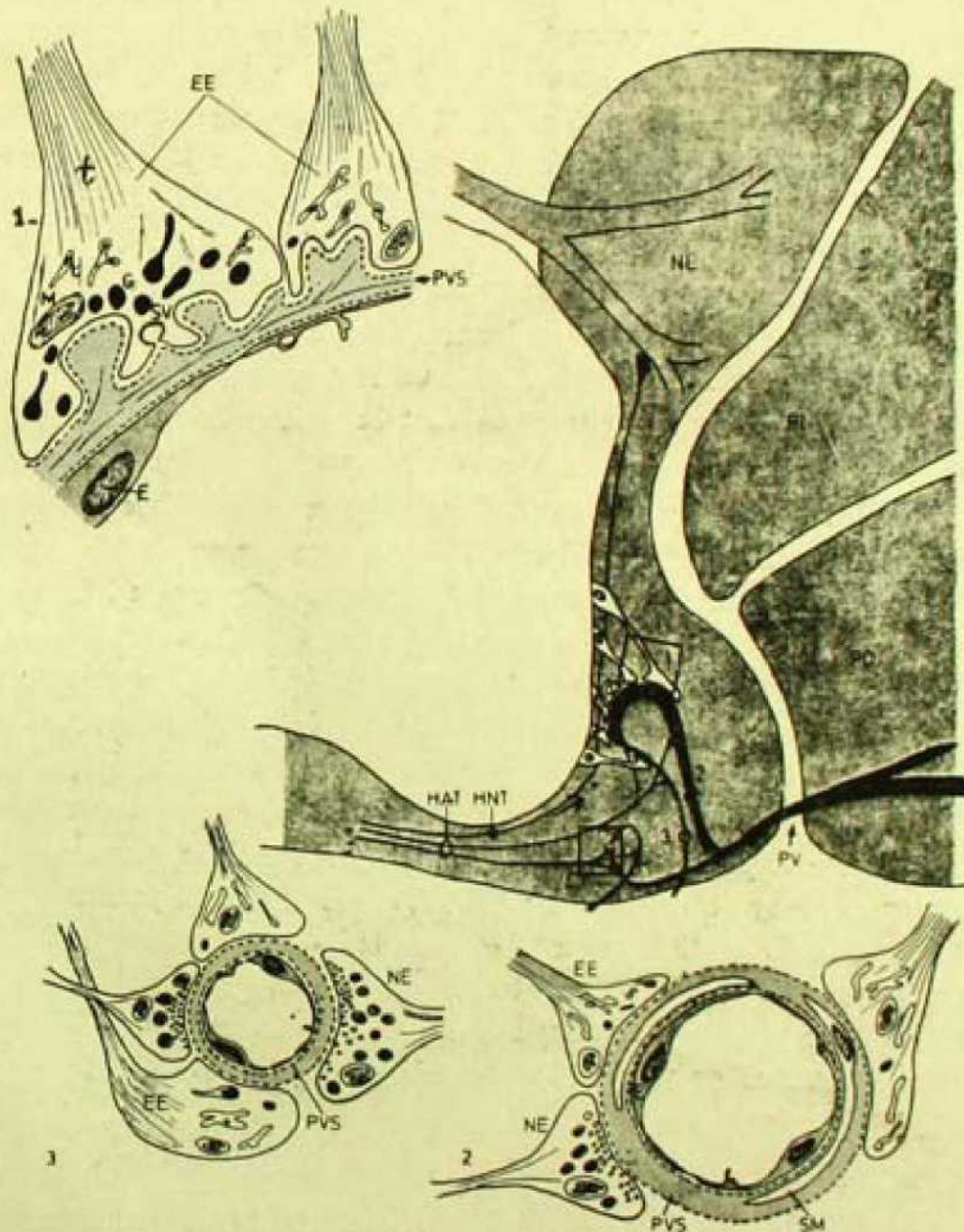


Fig. 12.2. Diagram showing the interrelationships between the ependyma of the toad median eminence, the short and long capillary loops of the hypothalamo-adenohypophyseal portal system and the nerve

*Continued to next page—*



Depending upon the ultrastructure of the basal processes, the tall ependymal cells are of two types. Processes of both the types are filled with filaments and tubular formations (smooth endoplasmic reticulum). The fundamental difference between the two types is that in one type the tubular formations are partly filled with an electron dense material and these tubular formations have connexion with electron dense granules of different shapes and sizes at the ending of the ependymal processes. In the other type of basal process these tubular formations are electron lucent (Rodriguez, 1969; Scott and Knigge, 1970; Knigge and Scott, 1970). The surface of these ependymal endings is irregular and intermingles with the rough surface of the perivascular basement membrane of the long and short loops (fig. 12.2).

Exogenous peroxidase injected into the CSF is incorporated into most of the ependymal cells of the toad median eminence. Cells with no basal processes do not incorporate the peroxidase. The peroxidase has no entry into the intercellular spaces between the ependymal cells. Different behaviour has been noted in the neighbouring ependymal cells. Little or no peroxidase has been noted to be incorporated in the ependymal lining of the neural lobe. Peroxidase passes through the intercellular spaces of the ependymal lining in the region of the infundibular nucleus and accumulates surrounding each neuron or is contained in some glial cells. Rodriguez (1972) suggests that at the level of the infundibular nucleus the encephalo-CSF barrier is not present. "The histological study clearly shows that most ependymal cells of the median eminence are interposed between the CSF and the portal capillaries. The ultrastructure of these cells strongly points to the possibility that they may perform a transport function. The fact that these cells may absorb exogenous peroxidase present in the CSF could be taken as an indication that transport may occur from the CSF towards the basal processes. Transport in the opposite direction cannot be excluded. The presence of ZIO (Zinc iodide-osmium tetroxide impregnation technique)-positive material within tubular formations of these cells might indicate that some of the substances being transported are monoamines or related compounds"—(Rodriguez, 1972).

*Contd. from page 247*

tracts entering the hypophysial region. (HNT), Hypothalamic-neurohypophysial tract; (HAT), hypothalamic-adenohypophysial tract; (NL), neural lobe; (PI), pars intermedia; (PD), pars distalis; (PV), portal vein. The ultrastructure of the areas indicated in square (1) and circles (2) & (3) is sketched in schemes 1, 2 and 3, respectively. A drawing of the area framed in square (4) is shown in figure 12.1. (EE), ependymal ending; (NE), nerve ending; (M), mitochondrion; (G), granule; (SV), spinous surfaced vesicles; (PVS), perivascular space; (E), endothelium; (SM), smooth muscle cell?; (f), filaments; (t) tubules. From Rodriguez (1972). Courtesy of S. Karger AG, Basel.



Special nervous elements connect the CSF to the portal vessels (Rodriguez, 1972). They are situated in the infundibular nucleus-median eminence region. The cells are located either in the ependymal layer or immediately beneath this layer and have a thick and short process which protrudes into the third ventricle and a long process which proceeds ventro-laterally to end on the portal vessels. This vascular process near the cell body is thick and the rough surface has projections which resemble dendritic spines. More distally the process is smooth and thin.

The special type of bipolar cells are located in the floor and in the ventral end of the lateral walls of the infundibular recess. The author could not decide whether these cells are neurons, ependymal cells or a special nervous element; but it is definite that they are in some way involved in the hypothalamo-adenohypophysial interrelationships.

A receptor cell in the toad hypothalamus was found by McKenna and Rosenbluth (1975) by Golgi impregnation method. The infundibular recess of the brain of *Bufo marinus* is lined by a population of catecholamine-containing cells. By electron microscopic and fluorescence histochemical study it was thought that the cells with an apical process to the ventricle and presynaptic to neurons play a sensory role. These cells relay information about the CSF to neurons at the somatodendritic synapses. The authors characterize these cells further by Golgi impregnation studies. An apical process projects from the cell into the ventricle where it comes in contact with the CSF. Rather thick (0.6 to 1.2  $\mu$ m) processes proceed from the basal pole of the cells. The processes are rough in appearance and branched and they course into the underlying tissue. Lateral processes may be thick or thin, the thicker ones resembling the basal processes. They do not branch and show varicosities at regular intervals. EM study of the Golgi-impregnated cells demonstrates the presence of axon terminals along the basal processes. It proves conclusively that these processes serve a dendritic function. "Thus, the infundibular subependymal cells, which have characteristics of receptor cells, also possess a system of dendrites. Axon terminals found along these dendrites, as well as the terminals found along the apical process and cell body described in the earlier study, serve most likely to modify the sensitivity of the sensory cells".

Oksche and Hartwig (1975) while studying photoneuroendocrine systems and the third ventricle found that these systems contain aminergic pathways to the intermediate lobe and they are involved in colour changes (inhibition of the intermediate lobe). In *Rana temporaria* these pathways could be traced back to the CSF-contacting neurons of the paraventricular and preoptic recess organs. The particular part of the diencephalic primordium i.e. its ependymal matrix, is capable of formation of photosensitive cells. Lamellar structures are not obligatory for photoreception. Photoactivation of an enzyme represents the starting step in a model for biological photoreception. "In poikilothermic vertebrates, the pineal organ mediates paling in darkness. The light receptor-function of



anurans, teleosts, elasmobranchs, and lampreys has been well established with electrophysiological methods (Dodt). In anurans, it has been shown microspectrographically that the outer segments of the pineal photoreceptor cells contain photopigments (Hartwig). Microspectrofluorimetric studies indicate that these receptors also contain an agranular pool of 5-HT or 5-HTP. In pineal sensory cells, an input of photic information might be converted into both electrical and neurohumoral outputs. In several species of poikilothermic vertebrates, the pineal organ is hollow and in connexion with the third ventricle. Thus, the outer segments of the receptors are bathed in CSF".

### *The amphibian neural lobe*

Rodriguez(1971) observed four types of endings in the neural lobe of all the specimens of *Bufo bufo*. A fifth type could be recognized in some. Analysis of the Kolmogorov-Smirnov test to the axons showed that there are at least four types of axons in the neural lobe of the toad. They are : typeII has granules of about 100nm mixed with numerous small clear vesicles of 40nm.

TypeIII has granules of moderate electron density with a diameter of 150-180nm.

Type IV has granules denser and smaller than previous ones (about 130nm).

TypeV fibres could be found only in some specimens. They contain 250nm granules of low electron density.

TypeI contains only small vesicles.

TypesI and II are very rare. TypesII, III, IV, V fibres were noted in the hypothalamo-hypophysial tract at the level of the infundibulum.

In the frog (*Rana pipiens*) typeV fibres were never observed. Rodriguez and Dellmann(1970) transected the hypothalamohypophysial tract before it entered into the median eminence. An accumulation of neurosecretory granules in the proximal stumps of the divided axons could be found. Axons were loaded with several hundreds of granules. Axons of typesIII and IV belong to *neural lobe fibres*. TypesI and II are *median eminence fibres*. Some of the type II fibres might be neural lobe fibres.

In the neural lobe of the amphibian species (toads and frogs) the *ependymal component* is not so well developed. The small number of ependymal cells are connected to a few subependymal capillaries. Highly developed system of tubules and large and dense mitochondria have been noted in these ependymal cells. Pinocytotic vesicles were observed to open (apically) into the ventricle and basally towards the perivascular space. Apical microvilli were absent. Pituicytes are not plenty and they are mostly present at the hilar region of the neural lobe. Very rarely they have contact with the perivascular basement membrane.

The connective tissue cells are usually situated in the perivascular spaces. Mast cells, plasma cells and fibroblasts were rarely observed in the neural lobe.



In the amphibians granules of 150-180nm in diameter carry arginine vasotocin (AVT). Granules of 130nm store mesotocin. Pale granules of 250nm could be observed by Rodriguez(1971) but its nature could not be ascertained. Rodriguez(1970) stressed the possible importance of an interrelationship between the CSF and neural lobe blood through the ependyma.

Jorgensen, Rosenkilde and Wingstrand(1969) studied the role of preoptico-neurohypophysial system in the water economy of the toad *Bufo bufo* L. The effect of neurohypophysectomy or of lesions in the preoptic region of the hypothalamus on water balance was studied in the toad. There were few or no surviving cells in the preoptic nuclei of the lesion-bearing toads and the nsm in the pars nervosa was completely reduced or totally absent. The operations had little effect on the antidiuretic response to moderate dehydration or on the increased cutaneous water permeability caused by severe dehydration. No correlation between the response to dehydration and the completeness of elimination of the preopticoneurohypophysial system could be observed. An attempt was made by the authors to delineate the role played by the preopticoneurohypophysial system in the water economy of the toad. "It seems that the antidiuretic response to an osmotic load and an increased permeability of the urinary bladder to water may largely depend on a functioning preopticoneurohypophysial system. In the absence of the neurohypophysis the system appears unable to exert its functions in the water economy of the toad".

#### *Morphological cell types of the amphibian pars distalis*

van Oordt(1963) reviewed earlier works and came to the conclusion that in anurans and urodeles there are five cell types in the pars distalis. Cordier(1953) found one cell type which was carminophilic, orangeophilic, and PAS-negative and two types of PAS-positive cells in the pituitary of *Xenopus*. One type of PAS-positive cell had cyanophilic inclusions and they were located in the centro-ventral area of the pars distalis in small groups. The other PAS-positive cells were also orangeG-positive and evenly distributed. Cordier and Herlant(1957) further extended their studies by other histochemical methods. Saxen *et al.*(1957) also noted three cell types. A third PAS-positive cell was found by van Oordt (1961) in the rostral tip of the distal lobe. The chromophobic cells in this area had fine granules which were weakly PAS-positive and orangeG-positive. Four cell types were described by Guardabassi and Bianchi(1962) and Srebro(1964) in the pituitary of *Xenopus laevis*. Doerr-Schott(1965) identified one acidophil cell type and three types of basophils. In *Xenopus laevis* Kerr(1965) described five morphological cell types and also undifferentiated chromophobes. Fixatives used were Bouin's fluid or Bouin-Hollande-sublimate. Staining methods were PAS-orangeG in combination with Luxol fast blue or AB and AF. Kerr(1965), van Oordt(1968), Holmes and Ball(1974) and other authors described the cell types in greater details.



*Acidophils type1 (A1)* : The cells in *Xenopus* are large and well granulated. They stain with orange G, erythrosine and Luxol fast blue. They are PAS, AB, AF-negative. These cells are distributed in different parts of the gland but maximum number of them have been noted in the rostro-central area (Kerr, 1965). In *Rana* and *Bufo* these cells are found to be scattered (van Oordt, 1968). The A1 cells were found to be erythrosinophilic in *Rana temporaria* and *Rana esculenta* by van Oordt, van Dongen and Lofts (1968) and Rastogi and Chieffi (1970). In *Bufo vulgaris* L, A1 cells are erythrosinophilic and carminophilic (Zuber-Vogeli, 1953; van Oordt, 1966; Mira-Moser, 1969). Ortman (1961) found A1 cells in *Rana pipiens* to be orangeG-positive. These cells are PAS-negative. van Kemenade (1969) found these cells to be weakly lead haematoxylin-positive.

A1 cells are carminophilic in *Necturus* and orangeG-positive in *Triturus marmoratus*, *T. cristatus*, *T. viridescens*, *Taricha torosa*, and *Pleurodeles waltlii* (Pasteels, 1960; Dent, 1961; Doerr-Schott, 1966; Mazzi *et al.*, 1966; Doerr-Schott, 1968).

Gabe (1972) noted a single acidophil in the apodan *Ichthyophis glutinosus*.

Ultrastructurally A1 cells had granules varying from 200 to 500nm in diameter in anurans and 250-550nm in diameter in urodeles (Doerr-Schott, 1968, 1971; Clauss and Doerr-Schott, 1970).

*Acidophils type2 (A2)* : These cells are densely granulated. A2 cells in *Xenopus* are smaller than the A1 cells. A2 cells have slight affinity for PAS. In Herlant's AB-PAS-orangeG the acidophils type1 are yellow but the A2 cells are light brown or orange in colour. A2 cells are concentrated in dorso-caudal part of the pars distalis and seen to be scattered throughout the entire caudal part. In *R. temporaria* A2 cells are lead haematoxylin-positive (van Kemenade, 1969). They may be transformed into chromophobes (Kerr, 1965).

Acidophils type2 can more easily be distinguished in bufonids than in *Xenopus*. They are situated in groups of medium sized orangeophilic and faintly PAS-positive cells lining the blood vessels in the dorsocaudal part of the pars distalis (Zuber-Vogeli, 1953; van Oordt, 1966; Doerr-Schott, 1968; Mira-Moser, 1969). In the viviparous toad, *Nectophrynoides occidentalis* these cells are found in groups forming palisades along capillaries in the dorsocaudal part of the pars distalis. They are erythrosinophilic and are found only during the first part of gestation (Zuber-Vogeli, 1968). These cells may even be absent in part of the year.

Two types of acidophils have been reported in the urodelan adenohypophysis by most authors. Dorsocaudally located A2 cells are well developed. This resembles what has been observed in *Bufo*. Pasteels (1957, 1960) observed first a definite PAS-positivity in A2 cells. Mazzi *et al.* (1966) visualized five morphological cell types having same distribution pattern as in anurans in *Triturus cristatus carnifex* using more modern methods. Doerr-Schott (1968) found A2 cells to be erythrosinophilic and arranged like a palisade around capillaries in *Triturus*



*marmoratus*. A2 cells of other urodeles are carminophilic and erythrosinophilic (Pasteels, 1960; Doerr-Schott, 1966, 1968).

Ultrastructurally the granules in A2 cells are 100-300nm in diameter in anurans and 100-250nm in urodeles (Doerr-Schott, 1966, 1968, 1971; Clauss and Doerr-Schott, 1970).

**Basophils type1 (B1):** These cells are large, often vacuolated with very strong cyanophilic, PAS, AB, and AF-positive granules. They are orange G-negative and found in small groups in the centroventral region of the pars distalis of *Xenopus*. B1 cells in bufonids are relatively small and often only a few isolated cells or small groups of them can be observed in the centroventral part or elsewhere in the gland (van Oordt, 1968). Four morphological cell types could be found in the common frog, *Rana temporaria* by van Oordt (1961, 1962, 1963) and Doerr-Schott (1963). These four cell types resemble A1, B1, B2, B3 cells of *Xenopus* and *Bufo*. Purely cyanophilic B1 cells without any OG-positive inclusion in adult frogs are difficult to find out. Small B1 cells could be found scattered in the pars distalis and their concentration in the centre of the lobe is uncertain (Ortman, 1961; van Oordt, 1968; Rastogi and Chieffi, 1970). Zuber-Vogeli (1968) found the B1 cells to be scattered in the pars distalis of *Nectrophrynoides*.

In normal untreated newts B1 cells are very difficult to find out. This is because of their close resemblance to B2 cells without orange inclusions and also because they are very weakly chromophilic. Mazzi *et al.* (1966) found that B1 cells of *Triturus cristatus carnifex* could easily be recognised and were plenty in number after thyroidectomy. In *Triturus cristatus carnifex*, *Salamandra atra* and *T. viridescens* B1 cells can be easily recognised only at metamorphosis and after thyroidectomy (Copeland, 1943; and Dent, 1961).

Gabe (1972) identified typical B1 cells in *Ichthyophis*.

Ultrastructurally, these B1 cells had spherical or elongated granules. The size-range-distribution was 120-500nm in anurans (Doerr-Schott, 1968; Clauss and Doerr-Schott, 1970; and Mira-Moser, 1970). In the urodele *Triturus (Notophthalmus) viridescens* Cardell (1964) and Dent and Gupta (1967) noted the B1 granules to be 180-250nm in diameter.

**Basophils type2 (B2):** These cells are present in large numbers throughout the pars distalis. Rostrally situated cells are larger than the caudal ones. They differ from B1 cells regarding staining properties in two important points. After staining with PAS-orangeG, they become brown instead of red-purple due to an affinity for orangeG. With Azan the cells are carminophilic and not purely cyanophilic. They can be stained also with Luxol fast blue and azofuchsin. The granules are only AF+ in sections strongly oxidised with acid permanganate (Gabe's variant) but not when the staining is preceded by a mild oxidation in Lugol's solution (Halmi's variant).



The B2 cells in bufonids contain finer and larger granules and globules. They not only stain with PAS and AB but are also orangeophilic, azocarmineophilic and azofuchsinophilic. Most of the smaller granules are exclusively cyanophilic (van Oordt, 1968).

In *Rana temporaria* two types of inclusions have been noted in B2 cells after staining with trichrome methods (classical). They may be small cyanophilic granules or smaller and larger orangeophilic elements. Majority of them are stainable with PAS, Gabe's AF, and Herlant's AB. Some of the larger, orangeophilic globules are AF and AB-negative. Varying quantity of orangeophilic granules and globules is noted. It is difficult to distinguish between B1 and B2 cells in pituitaries where B2 cells are predominantly cyanophilic (van Oordt, 1968).

Acid phosphatase was present in large granules and sometimes in smaller granules of B2 cells of *R. temporaria* and *Bufo bufo* by using cytochemical techniques adapted for EM (Doerr-Schott). Other granules large or small did not contain acid phosphatase. She therefore thought that B2 cells produce two types of granules. One type is secretory granule and the other type is lysosome. Kiecken, Nunn, Wachtler and Pearse (1965) observed acid phosphatase in B2 cells of *Triturus cristatus carnifex*. Acid phosphatase was not contained in other cell types, smaller granules and organelles in B2 cells. The globules containing acid phosphatase are lysosomes. Wachtler and Pearse (1966) found five lysosomal enzymes in the large spherical granules of B2 cells in *Salamandra S. salamandra* and in *Triturus cr. cristatus*. These acid hydrolases were acid-phosphatase,  $\beta$ -glucosaminidase,  $\beta$ -glucuronidase, sulphatase and E600-resistant esterases. Holmes and Ball (1974) stated that the globules resemble the R granules of teleostean gonadotrophs and they are apparently lytic bodies like the R granules.

van Oordt (1961) thought that B2 cells of *Rana esculenta* are same as in *Rana temporaria* regarding the granules and globules. Ramaswami (1962) observed same four cell types in *Rana cyanophlyctus*. Interpretation in *Rana pipiens* is difficult. Ortman (1956, 1960, 1961) described five cell types and undifferentiated chromophobes. The OG cells and purple cells correspond to A1 and B3 respectively. The AF cells and the aniline blue cells with numerous red granules together form B2 cells. Aniline blue cells with few or no red granules partly correspond to B1 and partly to B2 cells (van Oordt, 1968).

In *Triturus vulgaris* (Koscielski, 1956), *Triturus viridescens* (Dent, 1961) and *Triturus marmoratus* (Doerr-Schott, 1965, 1966) B1 and B2 cells could not be differentiated into two different cell types.

In the apodan *Ichthyophis* B2 cells are situated in the lateral pars distalis.

Ultrastructurally B2 cells of *Rana*, *Bufo* and *Xenopus* contained polymorphous granules and globules of moderate electron density. Their diameter varied from 100 to 900nm (Doerr-Schott, 1962-1966). In *T. marmoratus* and *T. cristatus* B2 cells had spherical granules of 160-320nm in diameter and large, membrane-bound



osmiophilic formations containing entwined bundles of parallel tubules. Cardell (1963, 1964) observed that B2 cells in *Triturus viridescens* contained small irregular granules 120 to 320nm and large irregular globules of 2,000nm in diameter. Doerr-Schott(1966) noted similar structures in B2 cells of *T. vulgaris* (van Oordt,1968).

**Basophils type3 (B3) :** In *Xenopus* these cells are small, cubical or elongated having fine PAS and orange G-positive granules. These basophils are AB-negative. The granules are AF-positive (Halmi's procedure). Trichrome methods impart a greyish blue or lavender blue colour. There are two characteristics of B3 cells. (a) They are concentrated in a crescent-shaped zone round the rostral border of the pars distalis; (b) These cells are associated with blood vessels entering the anterior third of the distal lobe from the median eminence (Kerr,1965). van Oordt(1968) said that in bufonids the B3 cells are not concentrated in the anterior process but they line the blood vessels coming from the median eminence and entering into the pars distalis. B3 cells are concentrated in the rostroventral region and small groups may occur in the central area and other parts of the gland. Holmes and Ball(1974) said that B3 cells are essentially amphiphilic. Typical B3 cells are PAS, AF (without strong oxidation) and aniline blue-positive on the one hand and on the other hand they are more or less stainable with orange G, azocarmine, acid fuchsin and others. B3 cells have been noted in *Rana temporaria*, *R. pipiens* and *R. cyanophlyctis* by Ortman(1961) and van Oordt(1968). In *Rana esculenta* B3 cells behave like acidophils(Dupont,1967, 1968; Dupont and Peltier, 1970) and they have been termed so. These cells are faintly PAS+ and strongly orangeG-positive and erythrosinophilic. They are orange with PAS-OG and red after Herlant's Alizarin blue tetrachrome. They have some affinity for Alizarin blue and after Herlant's Alizarin blue they look purple-violet. Dupont (1971) found these cells in pituitary grafts to have less affinity for orangeG and are more strongly PAS-positive.

B3 cells have strong affinity for MacConail's lead haematoxylin (van Oordt; 1968; van Kemenade,1969).

B3 cells have been noted in the pars distalis of *Pleurodeles waltlii* (Pasteels, 1957, 1960), *Salamandra salamandra taeniata* (Joly,1959) and in *Salamandra atra* (Mazzi *et al.*,1966).

The basophils type3 of *Triturus cristatus carnifex* are strongly AF+ in unoxidized or oxidized sections (van Oordt,1968). Similar reaction was noted by Doerr-Schott(1966) in *T. marmoratus*. In *Salamandra atra* (Mazzi *et al.*,1966) the B3 cells are less chromophilic. In *Necturus* the B3 cells are extended more laterally and caudally in comparison to other amphibians. They are situated lining the sinusoidal blood spaces (Aplington,1942, 1962) and are PAS + and AF-positive (after mild oxidation with Lugol's solution). They are purple after Masson's trichrome staining.

In the apodan *Ichthyophis* B3 cells are situated rostrally. The cells are violet after Herlant's Alizarine blue tetrachrome; strongly PAS-positive and AF-negative after permanganate oxidation (Gabe,1972).



Ultrastructurally B3 cells have small secretory granules of variable electron density. They are 100-200nm in diameter in *R. temporaria* and *Bufo bufo*, 100-220nm in *Xenopus*, 130-240nm in *Bombina variegata*, 150-300nm in *Triturus marmoratus* (Doerr-Schott, 1968; Clauss and Doerr-Schott, 1970).

Protein-bound sulphydryl (SH) and disulphide (SS) groups are found only in the acidophils and lysosomal bodies of B2 cells (van Oordt, 1968; Cordier and Herlant, 1957; Gabe, 1958; Goos *et al.*, 1966; Pasteels, 1960). Other histochemical reactions have been dealt with very nicely by van Oordt (1968).

*Stellate cells*: Holmes and Ball (1974) discussed in detail about this cell type in different vertebrates. These virtually agranular cells are situated also in the pars distalis of amphibians. They have long fine processes which pass between and contact with the endocrine cells. The cells are linked to each other and to the endocrine cells by desmosomes. The processes of the stellate cells are connected with the connective tissue capsule of the pars distalis and also form end-feet against the outer basal membrane of pericapillary spaces in the gland (Cardell, 1964, 1969; Masur, 1969; Bunt, 1969; Compher and Dent, 1970). Holmes and Ball state that their function is not definite in amphibians. Dent and Gupta (1967) observed that these cells appear first in the young larva of *Triturus viridescens* and they increase in number and the processes become longer during metamorphosis and terrestrial life. Sustentacular function was however attributed to this cell system by Cardell (1969). There are large fenestrations at frequent intervals. Chromophilic cells contact each other through these fenestrations. Holmes and Ball state that it is not likely that they are secretory as they have little RER, a small Golgi complex and few granules. A transport function to this cell system has however been attributed in vertebrates by Vila-Porcile (1972) and it has a role in the transport of material between blood vessels and endocrine cells.

The pattern of distribution of different cell types in a typical anuran pituitary is diagrammatically depicted in fig. 12.3 after van Oordt (1968).

#### *Cell types in pars tuberalis and pars intermedia*

In anurans and urodeles the *pars tuberalis* consists of small, poorly differentiated cells with little, weakly cyanophilic and PAS + cytoplasm. Secretory granules are usually absent. Occasionally acidophilic and basophilic cells from the pars distalis may be found in the tuberal lobe (van Oordt, 1968). Burlet and Legait (1967) observed in *Rana esculenta* that the volume of pars tuberalis decreases during growth and after hypophysectomy. Doerr-Schott (1971) studied the pars tuberalis of *Rana temporaria* ultrastructurally. There are chromophobic *reserve cells* and another PAS-positive glandular cells with strong secretory activity have also been found. Axons and endings could not be identified definitely but there is a possibility of contact between neurosecretory fibres and endocrine cells.

Fitzgerald (1979) reviewed the structure and function of the pars tuberalis of the vertebrate adenohypophysis. The cells of the pars tuberalis contain secretory



granules (anura, 100 to 300nm; urodela, 185 to 230nm; birds, 100 to 300nm; rat, 100-150nm; rabbit, 100nm; and rhesus monkey, 150-350nm). These have been represented by Fitzgerald(1979) in a tabular manner with references. The

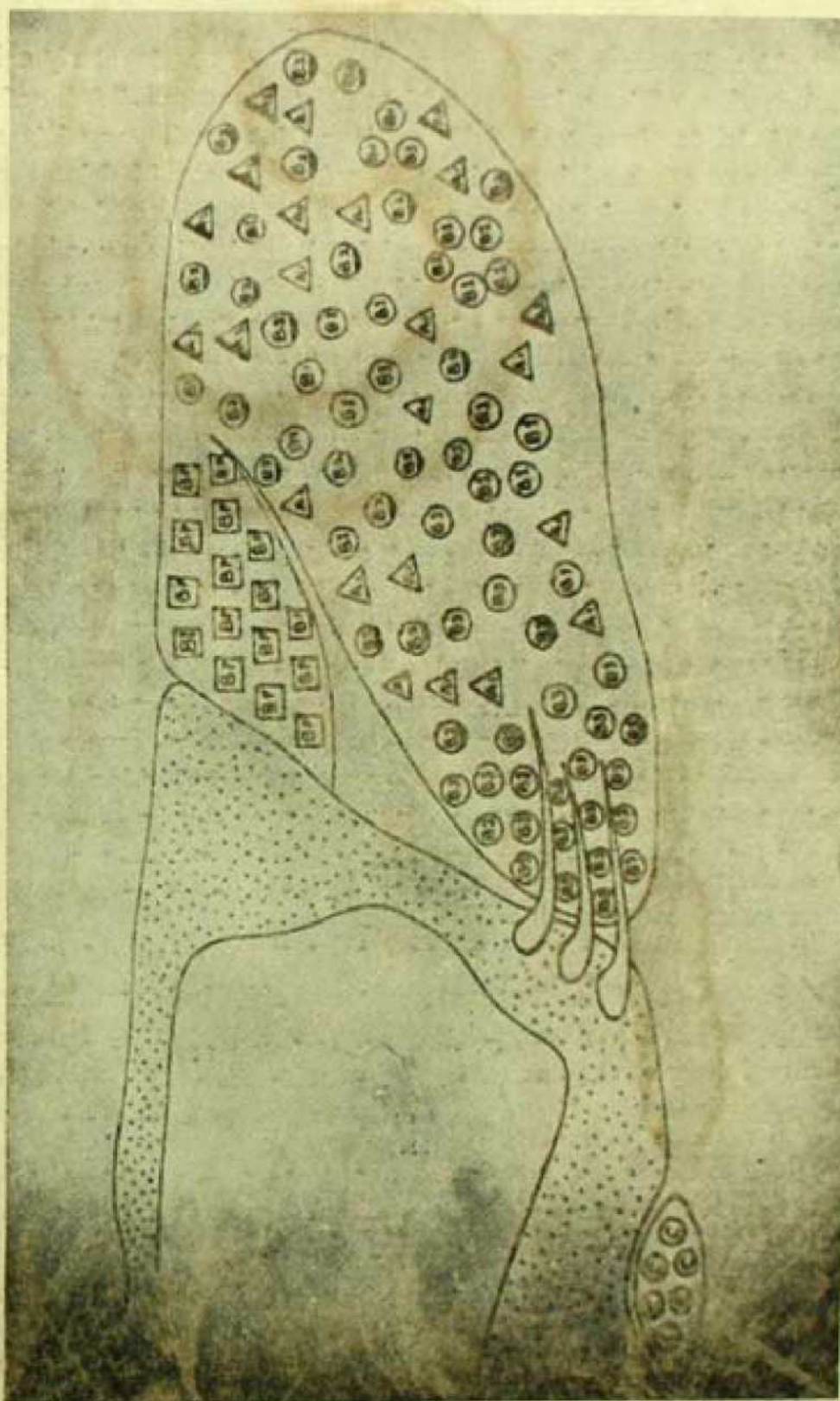


Fig. 12.3. Median section of the pituitary of an anuran showing the distribution of the cell types in the adenohypophysis. A<sub>1</sub> : acidophils type 1; A<sub>2</sub> : acidophils type 2; B<sub>1</sub> : basophils type 1; B<sub>2</sub> : basophils type 2; BP : basophils of pars intermedia; C : chromophobes of pars tuberalis.—From van Oordt (1968).

table also contains changes with sex, age, and season, immunocytochemistry, connections with tanycytes, and changes with hypophysectomy or castration. The findings that pars tuberalis cells contain plenty of secretory granules, the peculiarity of the vascularity of the part, presence of LH, FSH and TSH, seasonal ultrastruc-



tural changes, connections between PT cells and tanycytes, and certain other findings led Fitzgerald to think that this part of the pituitary gland is no more a nonfunctional lobe as was thought before. "PT may direct and regulate the function of the pars distalis or some other target organ."

*Pars intermedia* has one cell type. These are spherical or irregularly cuboidal and the nucleus is situated centrally. Cells lining blood capillaries look more elongated. The nuclei in these cells are situated in the basal part of the cell. The floccular cytoplasm has fine granules which are evenly distributed. In the frogs (Oreman, 1954, 1956; H. Legait, 1964), *Xenopus laevis* (Srebro, 1964), *Salamandra salamandra taeniata* (Joly, 1959), and *Triturus viridescens* (Dent, 1961) these granules are weakly PAS+ and less frequently they are strongly PAS+. Part of the PAS-positivity is due to glycogen in the cells and partly to the presence of mucoproteins and mucopolysaccharides (van Oordt, 1968). The granules are AF-positive after pretreatment with acid permanganate (Mazzi, 1954, 1959; Joly, 1959) and also when strong oxidation is omitted (Miller and Robbins, 1955; Ortman, 1956; Mazzi, 1959; Dent, 1961; Ortman *et al.*, 1966). The pars intermedia cells are lead haematoxylin-positive in *Xenopus laevis* and *Bufo bufo* (Mazzi, 1959). Cytochemistry of the intermedia cells has been discussed in details by van Oordt (1968). In addition to small granules there are also few large granules and vesicles of different size with a maximum diameter of 4.6 to 5.5  $\mu$  (Ortman, 1956). These vesicles are found to be accumulated between the cells which border the transverse vein of the intermediate lobe. They are commonly noted during strong secretory activity and contain mucoproteins, mucopolysaccharides, amino acids (tyrosine, tryptophan, arginine and cystine), lipids and phospholipids (van Oordt, 1968). They are AF-negative.

Weakly basophilic pars intermedia cells of *Necturus maculosus* can be transformed into fine granular erythrosinophilic cells (Aplington, 1942). In *Triturus* some A2 cells were observed in the pars intermedia. Two cell types (one dark and another light) were noted in *Xenopus laevis* and *Bufo bufo* by Mazzi (1959). *Light cells* are weakly PAS, AF, and lead haematoxylin-positive and are concentrated at the periphery of the narrow medial part of the lobe. The *dark cells* are smaller and strongly PAS, AF and lead haematoxylin-positive and are located in the wide lateral parts of the lobe and the centre of the medial part (van Oordt, 1968).

Ultrastructural study of the pars intermedia of *Bufo arenarum* was conducted by Iturriza (1964). Three cell types could be distinguished by their specific locations. It may be that these cells represent different stages of activity of a single cell type as intermediate stages could be found (Doerr-Schott, 1968; Hopkins, 1970; Doerr-Schott and Follenius, 1970). Saland (1968) studied the pars intermedia of the bullfrog (*Rana catesbeiana*) and *Rana pipiens* in varying conditions of background adaptation. Constant number of membranebound granules (2000 to 3000 Å) of varied shapes and densities were found in the predominant cell type of all glands. The rough ER of all dark-adapted animals and of bullfrogs in



transitional stages between light and dark adaptation shows striking whorl formation and expansion of cisternae. Plenty of dense, membrane-bound granules are associated with the Golgi region in these specimens. Glands from dark-adapted and transitional bullfrogs have many large (1-2 microns) dense droplets within ER cisterns. These are infrequently seen in dark-adapted *Rana pipiens*. They may be some form of polypeptide precursor to MSH. Increased protein synthesis in dark-adapted animals occurs when compared to the light-adapted ones. In each case there were nerve fibres and synaptic terminals having small, clear vesicles (200-300Å) and some dense core vesicles (600-1000Å) could be found throughout the pars intermedia. Evidences suggest that catecholamine mediated hypothalamic control for the pars intermedia exists. Neural inhibition from the brain first occurs at the level of hormone release and the control of synthesis is mediated by a feedback mechanism activated by amounts of hormone stored in the gland.

Stellate cells are found also in the pars intermedia (Hopkins, 1970; Nakai and Gorbman, 1969; Doerr-Schott and Follenius, 1970) (fig. 12.4). Glia-like supporting cells are found along the nerve fibres throughout the pars intermedia (Saland, 1968; Hopkins, 1971).

MSH is present in the membrane-bound granules which may be secreted by exocytosis (Hopkins, 1970). Weatherhead and Whur (1972) did not find all MSH to be contained in granules. The hormone may be associated with globular bodies within expansions of RER. This is a reserve store of the hormone (Doerr-Schott and Follenius, 1970) or Castell (1972) thought it to be a stage for rapid release. "The minor localization of fluorescent antibody to  $\beta$ 1-24 ACTH in the pars intermedia cells of several amphibians presumably reflects the structural overlap between ACTH and MSH (Doerr-Schott and Dubois, 1970, 1972). Antibodies to  $\alpha$  and  $\beta$ -MSH localize specifically in the pars intermedia cells in various amphibians (Doerr-Schott and Dubois, 1972)"—(Holmes and Ball, 1974).

#### Functional cell types

"Practically all publications dealing with the functional cell types in the adenohypophysis of Amphibia are based on the principle of parallel changes in the pituitary and its target organs"—(van Oordt, 1968).

#### Prolactin (LTH)

LTH is involved in the control of larval growth and detection of the same is by bioassay of the pituitary glands of anurans and urodeles (Bern and Nicoll, 1968; Nicoll and Nichols, 1971; Sage and Bern, 1972). Nicoll (1971) found *in vitro* secretion of LTH by pituitary glands. Of the acidophils only A1 cells are present in larval *R. pipiens* (Ortman and Etkin, 1963). Etkin and Ortman (1960) noted proliferation of acidophils in pars distalis of *Rana pipiens* larvae grafted in the tail. At the same time there was an acceleration of the larval growth (Etkin and Lehrer, 1960). Berman *et al.* (1964) and Nicoll *et al.* (1965) found that in *Rana catesbeiana* larval body weight and tail length cannot be accelerated by



mammalian STH, but mammalian LTH has this effect. STH could only stimulate the growth of the leg. Remy and Bounhiol(1966) observed giant *Alytes obstetricans* larvae by injecting bovine STH, LTH or both into hypophysectomized animals



Fig. 12.4. Pars intermedia of the bullfrog, *Rana catesbeiana*. Secretory granules may contain MSH. The nucleus shown in the corner is probably that of a stellate cell.—From Tsuneki (1977).  
 Courtesy of Professor Kobayashi and Dr. Tsuneki.

These diverse results seem to imply that the larval growth hormone in Amphibia differs from, but at the same time related to mammalian STH and LTH" (van Oordt, 1968).



Two types of acidophils are present in metamorphosed amphibians. In the tadpoles there is only one type of acidophil. Kerr(1966) thought that in *Xenopus laevis* acidophils in larvae dedifferentiate at metamorphic climax and become morphologically and functionally different after metamorphosis. van Oordt(1966) considered that larval acidophils might be identical with the dorsocaudal acidophils type 2 of the adult and they would be the source of LTH-like principle found in the pituitary of *Necturus maculosus* by Nicoll(1965) and Nicoll *et al.* (1966) and in the pituitary of *Rana temporaria* and *Rana esculenta* by A. Chadwick(1966). Copeland (1943) noted hypertrophy of cells resembling A1 in *Triturus viridescens* during the migration of red eft to water. This process is controlled by LTH(C. S. Chadwick, 1941; Grant and Grant, 1958).

With immunofluorescence technique Moriceau-Hay *et al.*(1979) could detect positive reactions to anti-LTH and anti-STH sera (prolactin and STH cells) in the pituitary of tadpole of *Xenopus laevis* D. In stage 42 positive reactions have been noted with anti-LTH sera and in stage 44 positive reactions have been obtained with anti-STH sera.

Ventral orangeophilic cells in the pituitary gland of *Pleurodeles* kept in a saline solution for 15 days appeared less active, dorsal erythrosinophilic cells enlarged and the intermediate lobe seemed to be less active than in freshwater animals (Olivereau *et al.*, 1977). Prolactin secretion is reduced in a saline environment. Somatotrophs and melanocorticotrophs are situated in locations as previously described using cytoimmunological data. The structure of the thyroid gland suggests increased activity in saline solution which may facilitate osmotic adaptation.

Actions of prolactin related to reproduction in amphibians are : water-drive (prior to reproduction), secretion of oviducal jelly, spermatogenic and/or anti-spermatogenic, and stimulation of cloacal gland development (Nicoll, 1974). Actions of prolactin on specific target cells or tissues have been summarized by Nicoll(1974) : tail and gill growth, limb regeneration, proliferation of melanocytes, structural changes accompanying water-drive, brain growth in tadpoles, spermatogenic, cloacal gland development and ultimobranchial stimulation. Prolactin acts on integumentary (ectodermal) structures in amphibians(Nicoll, 1974) : skin changes associated with water-drive, proliferation of melanophores, effects on toad bladder, and skin yellowing in frogs. Further actions involving synergism with steroid hormones or on organs also influenced by steroids are : stimulation of oviducal jelly secretion (estrogens and progestins),  $\text{Na}^+$  transport across anuran bladder (aldosterone), water-drive structural changes (sex steroids), spermatogenesis (androgens), and cloacal gland development (androgens) (Nicoll, 1974).

#### *Somatotrophin or growth hormone (STH)*

Growth hormone is generally thought to be formed in acidophils. Close correlation exists between the development and activity of the acidophils and growth in larvae and juvenile animals (Cordier, 1953; Saxen *et al.*, 1957; Pasteels,



1957, 1960; van Oordt, 1963, 1966; Kerr, 1966). Ortman (1961) and Ortman and Etkin (1963) found in *Rana pipiens* the A1 cells (LTH) to be present in the larva and the A2 cells to appear only after metamorphosis. This indicates that A2 cells are the source of STH. This is also proved in other anurans where A2 cells appear later after metamorphosis (van Oordt, 1966; Kerr, 1966; Zuber-Vogeli and Bihoues-Louis, 1971). These observations speak in favour of A2 cells being the source of adult STH at least. Holmes and Ball (1974) said that as A1 cells are the source of LTH in urodeles, A2 cells most probably secrete STH. However, separate existence of LTH and STH in urodeles cannot be definitely stated at present.

#### *Thyrotrophin, Thyroid stimulating hormone (TSH)*

B1 cells secrete TSH and B3 cells secrete ACTH. Grobstein (1938) observed degranulation and vacuolization in certain basophils in *Triturus torosus* after thyroidectomy. D'Angelo (1941) found a close correlation between the development and the activity of pituitary basophils and thyroids in larvae of *Rana pipiens*, *Rana sylvatica*, and *Rana palustris*. TSH secretion by basophils was also noted by Joel *et al.* (1949), Rugh (1953) and Cardell (1964). In *Triturus* Dent (1956, 1961) and Mazzi (1949, 1958) considered B3 cells as the source of TSH. Aplington (1962) ascribed B3 cells as the source of TSH secretion.

In *Rana pipiens* rostral half of the adenohypophysis does not contain more TSH than the caudal half and there are many indications to show that B1 cells are the source of TSH and not the B3 cells (Ortman and Lannen, 1963; Ortman, 1965; van Oordt, 1968). A correlation was found by Gasche (1946) between the activity of B1 cells and the thyroids in normal larvae during metamorphosis and in thyroidectomized and goitrogen treated animals. These findings were confirmed by Cordier (1953), Cordier and Herlant (1957), Saxen *et al.* (1957), Guardabassi (1961) and Goos and van Oordt (1967). Methylthiouracil in adult *Xenopus* led to hypertrophy of B1 cells (Guardabassi and Bianchi, 1962). Kerr (1965) observed degranulation, vacuolization and enlargement of B1 cells when thyroid function was blocked in adult *Xenopus* and these cells reduced in number on injection of thyroxine. Kerr (1966) thought that the activity of B1 cells correlated with the thyroid function in *Xenopus* larvae and B3 cells appeared shortly before metamorphic climax when gonad development starts. Similar observations were made by Pasteels (1954, 1957) in *Pleurodeles waltlii*, Joly (1959) in *Salamandra salamandra taeniata* and van Oordt (1966) in *Bufo bufo*. On severance of the connexion between the hypothalamus and the adenohypophysis of larval and adult *Pleurodeles waltlii* there was atrophy of the thyroids and reduction of B1 cells (Pasteels, 1954, 1957, 1960). Mazzi *et al.* (1966) finally concluded that hypertrophy of B1 cells occurred after thyroidectomy or thiouracil treatment and B1 cells should be considered as the source of TSH.

"Recently, one of the principal advocates of the view that the B3 cells are thyrotrophs has identified them as corticotrophs in several amphibians (Doerr-Schott, 1972; Doerr-Schott and Dubois, 1970, 1972)" (Holmes and Ball, 1974).



Schultheiss(1979) observed maintenance of growth and thyroid-stimulating properties of ectopic pituitaries in the Mexican axolotl (*Ambystoma mexicanum*).

Bullfrog LH acts as a *heterothyrotrophin* in a heterologous species (MacKenzie *et al.*,1978). The authors found evidence "that the thyrotrophin-receptor complex in the Reptilia has undergone extensive independent evolution such that its thyroid receptor preference responds to the LH rather than to the TSH from another class." The authors isolated TSH from the pituitary of the bullfrog (*Rana catesbeiana*) and the hormone could stimulate thyroid function in the homologous species. Bullfrog LH and FSH had little effect on the bullfrog thyroid. In heterologous species (turtle and lizard) bullfrog LH was most potent thyrotrophic factor, whereas, bullfrog TSH and FSH had little activity.

### *Corticotrophin (ACTH)*

Basophils type3 were considered previously to secrete LH or ICSH-like hormone (Pasteels,1957, 1960; van Oordt,1961; van Oordt and Lofts,1963; van Oordt,1963, 1965; van Dongen *et al.*,1966; Kerr,1965; Mazzi *et al.*,1966). Amphibian pituitary secretes only a single gonadotrophin.

ACTH cells in the anuran pars distalis are B3 cells (van Kemenade,1969, 1971; Larsen *et al.*1971). Metyrapone or aldactone stimulates B3 cells. The rostral half of the pars distalis containing B3 cells had ACTH activity. In *Xenopus* B3 cells were similarly distributed and ACTH activity was similar to that found in *Rana* (Peter Evennett and Lis Olesen Larsen—from Jorgensen,1976). Jorgensen (1976) reviewed the subject exhaustively.

Doerr-Schott and Dubois(1972) identified ACTH cells in different amphibians by immunofluorescence techniques. Fluorescence antibody against  $\beta$ 1-24 corticotrophin accumulated in B3 cells and to some extent in the pars intermedia cells of *Triturus marmoratus*, *Rana temporaria*, *R. esculenta*, *B. bufo* and *X. laevis*.

B3 cells in frogs, toads and newts are concentrated in the rostral pars distalis along the capillaries of the secondary plexus of the hypophysial portal system. These vessels determine the pattern of distribution of B3 cells. van Dongen *et al.*(1966) extirpated the pars distalis of *Bufo bufo* and regrafted it under the median eminence, wrongly oriented. After two months the B3 cells were seen to be normally distributed i.e. in the now-rostral part of the pars distalis into which the portal vessels enter. Similar redistribution of basophils were noted by Pasteels(1960) when the pars distalis was rotated through 180°.

Brands, Hansen and Jorgensen (from Jorgensen, 1976) studied the redistribution of basophils in the orthotopic autografted pars distalis of *Bufo bufo*. The gland was cut into a rostroventral part having majority of B3 cells and into a caudodorsal part. The rostroventral part was rejected and the caudodorsal part was regrafted under the median eminence. Secretion of ACTH was evidenced by normal moulting and survival of the graft-bearing toads. Plenty of B3 cells



appeared in the grafts. Maximum concentration of these cells were in that part where the portal vessels regenerated and entered into the graft. The authors suggest, "Presumably, a humoral gradient within the hypothalamic-hypophysial portal circulation determines the differentiation of the basophils<sup>3</sup> within the pars distalis of the toad."

Similar experiment conducted by Roy (unpublished observation) in *Bufo melanostictus* confirmed the previous findings and B3 cells were oriented maximally in that part of the caudodorsal graft where the portal vessels entered after regeneration. This procedure could maintain the plasma corticosteroid level to normalcy.

Ultrastructural studies of the pars distalis were conducted by Doerr-Schott (1972) in adrenalectomized frogs (*R. esculenta*) (cauterization). Examination was done 5-12 days after the operation. Adrenalectomy activated ACTH cells specifically and there was depletion of secretory granules with hypertrophic ergastoplasm and Golgi apparatus.

Metirapone treatment in the frog (*Rana temporaria*) led to cytological activity of ACTH secreting cells and adrenal cells (van Kemenade, 1969). The drug inhibits hormone synthesis in the adrenocortical cells as noted in mammals and thus decreases the negative feedback inhibition of ACTH secretion.

Stress increases plasma corticosteroids in the toad (*B. melanostictus*) (Roy, 1960) and in the frog (*R. temporaria*) (Jurani *et al.*, 1973).

*In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perfusion system was done by Delarue *et al.* (1979) to note the influence of ACTH upon aldosterone production. Ambient temperature and circulating levels of ACTH control aldosterone secretion. Aldosterone output is two times higher than corticosterone output in frogs. Aldosterone-corticosterone ratio is even larger after stimulation by high doses of ACTH. In the intermediate lobe of frog pituitary large concentrations of biologically active corticotrophin have been noted.

Vaudry *et al.* (1977) noted immunohistochemical changes in corticotrophs in the pituitary of *Rana esculenta* L after interrenalectomy and metopirone treatment. Fluorescent cells were situated (a) in the rostroventral part of the pars distalis around portal capillaries and (b) in almost all of the pars intermedia. A decrease in the number of pars distalis fluorescent cells (day 3) occurred after daily Metopirone injections and ultimately on day 7 they disappeared. The drug had no marked effect on the intermediate lobe fluorescence. There was decrease in the number of anti-ACTH binding cells 24 hours after adrenalectomy and after 4 days they totally disappeared. The number of anti-ACTH binding cells in the intermediate lobe was not changed. A polypeptide immunologically similar to mammalian ACTH is secreted by both the pars distalis and pars intermedia cells of frog pituitary.



### Gonadotrophin

Amphibian B2 cells secrete gonadotrophin. This has been reviewed by van Oordt(1968) and Holmes and Ball(1974). B2 cells are absent from the pituitary of larvae and they do not develop before the starting of the gonadal maturation (van Oordt,1968). B2 cells show seasonal changes coinciding with the annual gametogenetic cycle (van Oordt,1968). Castration leads to hypertrophy and degranulation of B2 cells in *Xenopus mulleri* (Cordier,1953), *Bufo bufo* (Zuber-Vogeli,1953), *Rana temporaria* (van Oordt,1961; Doerr-Schott,1963) and *Triturus cristatus carnifex* (Ferrerri and Peyrot,1962). Increase in the granulation of B2 cells in short term castration experiments with *Xenopus laevis* was observed by Kerr (1965) and B2 cells regressed after intraperitoneal implantation of testosterone pellet. With implantation of testosterone pellet in the dorsal lymph sacs of *R. temporaria*, van Oordt(1961) noted changes in B2 cells.

Total gonadotrophic activity is found throughout the pars distalis as B2 cells are similarly distributed (van Kemenade,1972) (Bioassay of fragments of pars distalis of *R. temporaria*).

B3 cells were thought to secrete LH previously (van Oordt,1968) but as now it has been observed that B3 cells secrete ACTH, and so B2 cells secrete FSH and LH. It is possible that amphibians may produce only a single gonadotrophin (van Oordt and De Kort,1969).

Fontaine and Burzawa-Gerard(1977) discussed the evolution of thyrotrophic (TSH) and gonadotrophic (GTH) hormones. The main hypotheses are: "A common ancestral molecule gave rise, by genic duplication, to two subunits  $\alpha$  and  $\beta$ . The first hormone ( $\alpha$ - $\beta$ ) was gonadotrophic but later had both functions (TSH and GTH). Duplication of  $\beta$  then gave rise to  $\beta_1$  (with gonadotrophic, LH-type, potentialities) and  $\beta_2$  (with thyrotrophic potentialities). Finally the FSH  $\beta$ -type subunit originated later from a duplication of  $\beta_2$ . Along these lines, hormonal specializations and diversifications occurred, due to genic modifications and associated structural changes in both  $\alpha$  and  $\beta$  subunits."

Daniels *et al.*(1977) conducted immunochemical studies on the pituitary gonadotrophins (FSH and LH) from the bullfrog, *Rana catesbeiana*. The anti-sera cross-reacted with the biologically active gonadotrophin molecules. FSH-like and LH-like activity in two gonadotrophic preparations could be separated from pituitaries of the leopard frog, *Rana pipiens* by Farmer *et al.* (1977).

Guha and Jorgensen(1978) studied the effects of hypophysectomy on structure and function of testes in adult toads, *Bufo bufo bufo* (L). They observed that gonadotrophin independence during the process of spermatogenesis is acquired late in the secondary spermatogonial phase. The seminiferous tubules disintegrated within about 2 months of hypophysectomy and connective tissue infiltrated the tubules. In the same year Guha and Jorgensen noted that in the highly dedifferen-



tiated testes 8 weeks after hypophysectomy, the trophic effects of HCG treatment could be distinguished.

### MSH

Pars intermedia has mainly one cell type and it is the source of MSH as has been proved by assay methods and biochemical techniques.

#### *Central nervous control of adenohypophysial functions*

Reviews in this field have been made by Jorgensen(1968, 1970, 1976), Dodd, Follett and Sharp(1971), and Holmes and Ball(1974).

#### *Control of LTH secretion*

LTH takes part in normal reproduction in *Triturus viridescens*. It causes transformation from the terrestrial to the aquatic stage which happens before reproduction (Grant and Cooper, 1965). Masur(1962) showed that ectopic transplantation of the pituitary in the terrestrial stage leads to the aquatic stage. "It appears that ectopic transplantation of the hypophysis induces the gland to secrete prolactin" Jorgensen(1968). Mazzi(1970) suggested that prolactin synthesis and secretion in the crested newt may be controlled by two factors: an inhibitory one (Prolactin inhibiting factor, PIF) and an activating one (Prolactin releasing factor, PRF). They are balanced and simultaneously produced by the hypothalamus. Release of PRF and PIF is thought to be controlled by temperature variations (Vellano *et al.*, 1968). Increased amount of LTH is secreted by the ectopically transplanted pars distalis in *Bufo bufo* (McKeown, 1972).

Clemons *et al.*(1979) studied the effects of mammalian thyrotrophin releasing hormone (TRH) on prolactin secretion by bullfrog adenohypophyses *in vitro* in short-term incubation and 24-hr organ culture experiments. Prolactin in the medium or in the incubated tissue was measured by either polyacrylamide disc gel electrophoresis and densitometry or by a homologous radioimmunoassay. Prolactin release could be effectively achieved *in vitro* in concentrations of 10ng/ml to 10µg/ml. Tissue prolactin content was found also to be increased. The authors concluded that TRH may function as a prolactin-releasing factor in the bullfrog.

Neurons of nucleus preopticus and preopticohypophysial tracts of various amphibia contain somatostatin or growth hormone inhibiting factor (SRIF) (Doerr-Schott and Dubois, 1978).

Kikuyama and Seki(1980) concluded that in bullfrog larvae the release of prolactin-like hormone from the pituitary gland is regulated by dopamine and that the receptor for dopamine exists in the pituitary gland. The effect of dopamine on the release of prolactin-like hormone from the adult bullfrog pituitary gland *in vitro* was also studied.



### Control of STH secretion

Little is known about the control of STH. Adult *Triturus cristatus* grows more rapidly in the grafted than in the control animals (Peyrot, 1966). Isolated urodele hypophysis produced normal amounts of growth hormone as evidenced by the normal growth rate of the animals (Jorgensen, 1968). Similar phenomenon was observed by Pasteels (1960) in adolescent and young adult individuals of *Pleurodeles waltlii* bearing ectopic transplants of the hypophysis. In the hypothalamectomized tadpoles of *R. pipiens* growth rate is normal (Hanaoka, 1967). Similar observation or even higher than normal growth rate has been obtained in this species with ectopic transplantation of the hypophysis (Etkin and Lehrer, 1960; Etkin and Ortman, 1960). Pehlemann (1962) observed normal growth rate in the tadpoles of *Pelobates fuscus* and *Rana esculenta* after ectopic hypophysial transplantation. McKeown (1972) measured plasma STH levels by radioimmunoassay in *Bufo bufo* and showed that ectopically transplanted pars distalis secretes very little hormone. The inference is that STH secretion is under stimulatory hypothalamic control (Holmes and Ball, 1974).

### Control of TSH secretion

Ectopic transplantation of the pars distalis strongly reduces TSH secretion as found out by the low rate of  $^{131}\text{I}$  uptake by the thyroid gland (van Dongen *et al.*, 1966). A number of observations on different urodeles show some degree of thyroid autonomy from the central nervous system (Mazzi, 1970). Jorgensen (1968) reported normal moulting in *Pleurodeles waltlii* bearing a pituitary autograft. Similar observations have been made by Jorgensen and Larsen (1963) for metamorphosed *Ambystoma mexicanum*, by Schotte and Tallon (1960) and Dent (1966) for several *Triturus* species. Thyrotrophic cells in the autotransplanted pituitary show hypertrophy after thiouracil (Mazzi and Peyrot, 1963). Moulting is not severely affected in the crested newt with chronic hypothalamic lesions (Mazzi, 1958). Mazzi (1970) stated that in several urodele species  $^{131}\text{I}$  uptake by animals bearing a heterotopic pituitary autograft is normal or almost normal as reported for *Ambystoma mexicanum* by Jorgensen (1968) and for *Triturus viridescens* by Dent (1966) or even higher than in controls as reported for *Triturus cristatus* by Peyrot *et al.* (1966).

Jorgensen (1968) concluded, "The evidence thus indicates that in adult urodeles the isolated hypophysis is able to secrete TSH in amounts necessary to cover normal daily maintenance requirements. During the periods of high TSH requirements, at metamorphosis, the TSH secretion of the isolated pars distalis falls short of the requirements. In anurans (toad, frog), however, the isolated pars distalis appears to secrete TSH at low rates both in larval and adult life".

Jorgensen (1970) found the rates of  $^{131}\text{I}$  uptake by the thyroids to be increased after transection of the hypothalamus in front of the optic chiasma, but it was low after ectopic transplantations of pars distalis. The experiments suggests that



the control of TSH secretion is complex and *perhaps includes inhibitory as well as stimulatory nervous components* (Rosenkilde, 1969).

In the frog, *Rana temporaria* transections anterior and posterior to the optic chiasma had no significant effect on the thyroidal  $^{131}\text{I}$  uptake, and this was high in both the groups (Rosenkilde from Jorgensen, 1970). Moreover, in contrast to the toad, the transplanted pars distalis exhibits pronounced autonomous thyrotrophic activity.

Voitkevitch (1961, 1962, 1965) observed that different anuran tadpoles do not metamorphose after extirpation of brain including telencephalon and preoptic region of the diencephalon in early stages, but tadpoles without telencephalon metamorphose normally. The author thinks that Gomori-positive neurons of the preoptic nucleus control the thyrotrophic function of the metamorphosing tadpoles. Weber (1965) attached similar importance to the magnocellular preoptic nucleus in the process of metamorphosis in *Salamandra salamandra* by brain cut experiments.

Goos and van Oordt (1967) described pseudoisocyanine-positive cells in the preoptic nucleus of *Xenopus laevis* which control thyrotrophic function of the pars distalis during metamorphosis. These neurons were distinct from the AF-positive magnocellular neurons. From the beginning of premetamorphosis the pseudoisocyanine-positive material increases in the dorsal part of the preoptic nucleus. Propylthiouracil administration in tadpoles leads to hypertrophy of TSH cells and the dorsal cells of the preoptic nucleus lose pseudoisocyanine-positive material.

The forebrain controls metamorphosis in *Xenopus laevis*. The removal of forebrain inhibited metamorphosis even when the diencephalon with the preoptico-neurohypophysial system was intact (Srebro, 1962).

Amphibian brain contains good amount of TRH (Reichlin, 1974; Taurog *et al.*, 1974). Taurog *et al.* (1974) could not find increase in TSH secretion by TRH acting on salamander or frog pituitaries (*in vivo* or *in vitro*). Jackson and Reichlin (1974) and Jackson (1978) found the hypothalamus of the rat, chicken, snake, frog, tadpole, and salmon to contain high concentrations of TRH. The level of TRH in amphibian hypothalamus was 3620 pg/mg tissue wet weight. The rat hypothalamus contained only 300 pg/mg tissue. *Pituitary-thyroid function is not stimulated by TRH in species lower than aves*. High concentrations of TRH is present in cerebral cortex (forebrain) of snake, frog, tadpole and salmon. These values are much higher when compared to the respective brain areas in the rat. It is 520 pg/mg tissue in the cerebellum of the frog and 165 pg/mg tissue in the salmon olfactory lobe. That these substrates are identical to TRH is evidenced by noting the parallel inhibition curves by immunoassay. Parallelism in inhibition curves with that of synthetic TRH was observed when the effect of dried methanol extracts of snake brain (cortex) and pooled extra-hypothalamic frog brain was studied on the inhibition of  $^{125}\text{I}$ -TRH binding with



anti-TRH. Extract of frog extrahypothalamic brain releases rat TSH *in vivo*. The increase is proportional to the ir-TRH (immunoreactive-TRH) content of the extract. The authors found ir-TRH in the circulation of *Rana pipiens*. It is chromatographically identical with native TRH and can release TSH in the rat (*in vivo* experiment).

In frogs TRH may be involved in the hormonal control of salt and/or water balance during hibernation and therefore, serve an osmoregulatory function.

Alteration in pineal and hypothalamic TRH produced by season, and the effect of illumination on pineal TRH content, support the view that TRH has a neuronal function in vertebrates, possibly as a neurotransmitter (Jackson *et al.*, 1977).

### *Amphibian metamorphosis*

Holmes and Ball (1974) summarized the observations of different authors including those of Etkin (1970). In amphibians there is hypothalamic stimulation for TSH secretion. For completion of metamorphosis TRF is probably always required. Low TSH secretion in the early larva is autonomous. In adult urodeles TSH secretion is mainly autonomous. In adult anurans TSH secretion generally depends on hypothalamic TRF. A hypothalamic inhibitory mechanism may also be present in them.

Etkin (1970) discussed the endocrine mechanism of amphibian metamorphosis. In anurans the metamorphic period has two distinct phases. There is rapid leg growth and certain other growth processes such as that of skin glands, in the first or prometamorphic period. In *Rana pipiens* this lasts for about 3 weeks at 25°C. This is followed by a shorter period of one week, called metamorphic climax. During this period the tail and gills are resorbed and the feeding apparatus is transformed. The length of the larval period is very variable.

Thyroid hormone (thyroxine,  $T_4$  or triiodothyronine,  $T_3$ ) induces the varied tissue changes. TSH controls the thyroid. Before metamorphosis thyroid activity was low and it increases progressively during prometamorphosis to achieve an extremely high level at the beginning of climax.

In the tadpoles of *Rana temporaria* the length of prometamorphosis is influenced by the following treatments: (1) thyroidectomy, (2) propylthiouracil, (3) l-thyroxine, (4) low temperature. The fluorescent pars distalis fibres persist until climax in normally developing tadpoles. The fibres persist until climax even when the prometamorphosis is prolonged artificially, irrespective of the reason. The fibres disappear when the prometamorphic period is artificially shortened or when a prolongation of prometamorphosis is interrupted by thyroxine induced climax (Stig Aronsson, 1978). "Thus the disappearance of the aminergic nerves is a metamorphic event associated with climax".



Norris *et al.* (1977) studied the thyroid function in pre- and postspawning neotenic tiger salamanders (*Ambystoma tigrinum*). The previously suggested antagonistic roles for thyroid hormones in larval amphibians with respect to spermatogenesis and follicular development have been supported by the observations of the authors. They observed a positive correlation between rising autumnal thyroxine levels and both spermiation and the later stages of follicle development.

Hypothalamic thyrotrophin releasing factor (TRF) passes through the pituitary portal vessels and activates pituitary TSH production at metamorphosis. "The median eminence of the hypothalamus differentiates its vascular area during prometamorphosis producing a well defined portal circulation by the beginning of climax". The development and activity of the median eminence cannot be a controlling authority for the starting of the metamorphosis but it is itself dependent upon the metamorphic stimulus. Maturation of TRF mechanism evidenced by the development of the vascular zone of the median eminence is stimulated by thyroid hormone. Thyroid activity is extremely low before metamorphosis. So it acts only imperceptibly on the tissues and the hypothalamus. This low level of activity acts in a positive feedback manner and starts prometamorphic change. In all species positive feedback build-up of the hypothalamic-pituitary-thyroid-axis occurs in prometamorphosis. "In this theory the *metamorphic clock* is turned on by the genetically-timed change in sensitivity in the hypothalamus. However, the rate at which the *clock runs* through metamorphosis is a function of the parameters of the positive feedback system."—(Etkin, 1970).

Etkin and Gona (1974) stated that the metamorphic pattern depends upon a pattern of thyroid activity. In premetamorphosis no effective level of hormone secretion has been reached. In prometamorphosis the initial level is very low but rises during this period to a very high level at the beginning of climax. The build-up of thyroid activity begins at stage XI, 20-25 days before climax. Two metamorphic transformations occur in the common American newt. "The first changes the aquatic larva to a terrestrial form called the *red eft*. This metamorphosis is comparable to anuran metamorphosis and, like that process, is induced by thyroid hormone. The second metamorphosis sends the eft back into an aquatic stage. This transformation can be induced by exogenous prolactin or simple pituitary transplantation." Prolactin-thyroid antagonism has been found to occur at some levels and synergism at others.

Prolactin-like hormone is the effective growth factor in tadpoles. This hormone inhibits the responsiveness of the tissues to  $T_4$  both *in vivo* and *in vitro*. Prolactin acts as a powerful and non-toxic goitrogen at high levels. "As pituitary grafts act like prolactin injections, the prolactin-thyroid antagonism appears to be physiological and not pharmacological."

Enemar (1978) observed that for normal growth rate and stimulation of growth by growth-promoting hormone(s), adenohipophysis is necessary in tadpoles of *Rana temporaria*. Hormone(s) seem to be secreted at the same rate as when the gland is removed from direct hypothalamic control.



Fractions were separated from pituitary glands of both larval and adult *Rana catesbeiana* by disc gel electrophoresis by Kikuyama *et al.* (1980). "Only a fast moving protein fraction had both a pronounced stimulating effect on the collagen synthesis in the tail fin of bullfrog tadpoles and a suppressive effect on  $T_4$ -induced resorption of *Bufo bufo japonicus* tadpole tails *in vitro*." Pigeon crop-sac test showed that this fraction had a pronounced prolactin activity and it was four times as potent as bovine prolactin in promoting collagen synthesis in the tadpole tail fin.

Etkin(1970) thought that the tadpole stage of development in the frog is under the influence of prolactin. It is produced autonomously by the pituitary in absence of hypothalamic control. Stimulation of growth is caused by prolactin and prolactin counteracts any thyroid influence which is present. Stabilization in the larval stage by the tadpole is thus achieved. At the beginning of prometamorphosis, hypothalamic mechanism is activated and thyroid hormone level is raised and this promotes metamorphosis. At the same time it cuts back on the action of prolactin and thus removes this brake to metamorphic change. It is not known whether the same hypothalamic factor activating pituitary-thyroid axis (TRF) also inhibits prolactin activity (PIF).

"There are evolutionary changes in the sensitivity of target organs to endocrine messengers and the acquisition of tissue responses which are specialized parts of the animal's adaptation to environment."

Dodd(1970) while discussing the paper of Etkin remarked that they measured TSH in the pituitary glands of *Xenopus* tadpoles in later stages of metamorphosis and they found peak TSH content at about stage 61. The most active stage of the thyroid gland as measured by histology and radioiodine uptake is stage 63. At stage 61, TSH is released from the pituitary more actively than it is synthesized. At stage 63 metamorphosis is almost complete except the presence of the tail. Tail resorption requires more thyroxine than any other event in metamorphosis. "Prolactin acts as a growth hormone in the completely metamorphosed young adults as well as in tadpoles."

Etkin and Gona(1968) could not find induction of metamorphosis in an anuran by TRH. Gona and Gona(1974) had similar observation in a urodele. Sister Wright *et al.*(1979) found that "prolactin antagonizes metamorphic changes of young tadpoles not generally considered to be at the thyroid-dependent stages of development."

Torok *et al.*(1979) studied the effect of prednisolone on the development of amphibian larvae and the antagonism of litoralon and prednisolone. Prednisolone enhanced the development of *Rana arvalis* Wolterstorffi larvae. Litoralon antagonized this effect. Glucocorticoid and thyroid hormones antagonize in some respects the effects of litoralon and vitamin A.



### *Control of Gonadotrophin secretion*

This has been reviewed by Jorgensen(1968, 1970), Mazzi(1970), Vijayakumar, Jorgensen and Kjaer(1971), Dodd, Follett and Sharp(1971), and Holmes and Ball(1974).

#### *Gonadotrophic centre*

Dierickx(1965, 1966, 1967) tried to localize the gonadotrophic centre by lesioning the base of the brain at various distances from the hypophysis in *Rana temporaria*. Line of section which was very close to the hypophysis inhibited the gonadotrophic function. Spermatogenesis and ovarian development was normal when the section passed just posterior to the optic chiasma. It left the middle and posterior hypothalamus intact and the anatomical relations to the hypophysis remained undamaged. When the level of section was between the optic chiasma and hypophysis, there was vitellogenesis and development of normal sized oocytes, but the ovaries were small. These experiments indicate the location of the *gonadotrophic centre* to be in the middle hypothalamus and it is of a diffuse nature (Jorgensen,1968). Dierickx(1965) thinks that the gonadotrophic centre is situated in the periventricular area of the pars ventralis of the tuber cinereum. Ovulation did not take place when the hypothalamus was sectioned just behind the optic chiasma in frog. For this purpose the preoptic nucleus is responsible. "At any rate the results indicate that the hypothalamic region involved in the control of gonadotrophic functions extends to the anterior region of the hypothalamus in the frog."—(Jorgensen,1968).

According to Dierick's hypothesis, higher centre controls the cyclical gonadotrophic activity in female Anura. A tonic gonadotrophin regulating centre is situated in the hypothalamus (Alpert *et al.*1976). LHRH is localized in the neurons of the septal area of frog's brain (*Rana pipiens* and *Rana catesbeiana*). It controls the cyclic gonadotrophin activity. Doerr-Schott and Dubois(1975) identified the localization of luteinizing hormone releasing factor in the brain of *Bufo vulgaris* Laur. Peptidergic LHRH septo-infundibular pathway passes underneath the preoptic recess or through the medial forebrain bundle. These fibres then course through the lateral infundibular hypothalamus and enter the median eminence from both sides. The tonic gonadotrophin regulating centre is situated in the nucleus infundibularis.

From experiments of Mazzi *et al.*(1974) it is found that the newt brain contains mammalian LHRH-like substance. Mazzi(1978) observed the effects of permanent lesions to the rostral preoptic area in the crested newt (*Triturus cristatus carnifex* Laur) on spermatogenesis. An arrest or a marked delay in spermatogenesis was observed. There are evidences which prove that telencephalo-hypothalamic connections are essential for seasonal spermatogenesis in this species.



Experiments reported by Callard *et al.* (1978) indicate that, "the conversion of androgen to estrogen and other neural metabolites by the brain is a primitive tetrapod characteristic and suggests that metabolism is an integral component of brain-steroid interactions which has been conserved during the evolution of vertebrates."

Brain areas outside the infundibular region are also of importance in the control of normal gonadal function in the female toad (*Bufo bufo*) (Jorgensen, 1968). The author found that the central nervous stimulation of hypophyseal gonadotrophic activity that is necessary for interstitial cell activity (maintenance of thumb pads) may be exerted by the infundibular region of the hypothalamus in the male toad.

Mazzi (1970) presented his observations in the crested newt (*Triturus cristatus carnifex* Laur). Development of spermatogenesis depends upon the integrity of the hypothalamo-hypophyseal connections (Mazzi, 1950, 1952; Mazzi and Peyrot, 1960, 1963). Damage to the preoptic region and the hypothalamic floor, insertion of a barrier in the median eminence and thereby preventing the regeneration of hypothalamohypophyseal tract and of the vessels coming from the hypothalamic floor, and heterotopic hypophyseal autotransplantation, are all followed by a marked loss of weight of the testis and there is blockage of spermatogenesis. Reactivation of spermatogenesis in animals with hypothalamic lesion is possible only when there is regeneration of portal vessels. Spermatogenesis is not possible with heterotopic pituitary autograft. Thus the vascular connections between the hypothalamus and the pituitary are very important.

The regressive phenomena in the testes of crested newt having permanent hypothalamic lesions or pituitary autograft are due to suppression of synthesis or release of gonadotrophin. FSH (B2) and LH (B3) cells are functionally inactive both at the optical and at the ultrastructural level.

There is a thermosensitive centre in the hypothalamus which is responsible for *thermo-pituitary-sexual-reflex*. "The hypothalamic thermosensitive centres operate like endocrine transducers in which chemical messengers such as FSHRF and LHRF are elaborated and transferred to the hypophysis through the hypophyseal portal system." Production of Follicle-Stimulating Hormone Releasing Factor (FSHRF) is increased by high temperatures and inhibited by low ones, while the reverse is true for Luteinizing Hormone Releasing Factor (LHRF). However, high temperatures depress LHRF but do not inhibit it (Mazzi, 1970).

Jorgensen (1970) found that neurons which take their origin in the posterior hypothalamus between the optic chiasma and hypophysis can maintain normal thyrotrophic and gonadotrophic functions.

Hypothalamus controls gonadotrophic activity of the pituitary (Goos, 1978). Stimulation of gonadotrophin secretion from incubated pituitaries of *Rana pipiens*



can be achieved by extracts of different areas of the brain of the same species and mammalian LHRH (Thornton and Geschwind, 1974). Induction of sperm release in frogs could be done by mammalian gonadotrophin-releasing hormone (Licht, 1974). Vellano *et al.* (1974) observed the effect of synthetic LHRH on ovulation in the crested newt. Mazzi *et al.* (1974) found gonadotrophin stimulation by chronic administration of synthetic LHRH in hypophysectomized pituitary grafted male newts. Alpert *et al.* (1976) localized LHRH in neurons in frog brain (*Rana pipiens* and *Rana catesbeiana*). LHRH-like system could be observed in the brain of *Xenopus laevis* Daud by immunohistochemical method (Doerr-Schott and Dubois, M.P., 1976). By immunofluorescence method LHRH has been localized in the forebrain and the neurohypophysis of the green frog, *Rana esculenta* L (Goos *et al.*, 1976). King and Millar (1979) investigated hypothalamic LHRH content in relation to the seasonal reproductive cycles of *Xenopus laevis*. Hypothalamic ir-LHRH of the frog (*Xenopus laevis*) is indistinguishable from synthetic mammalian LHRH. Variation of hypothalamic LHRH was observed by the authors in relation to season and reproductive physiological state in the frog. In sexually quiescent frogs collected in the nonbreeding season (winter) hypothalamic LHRH content was low. In reproductively active frogs collected in the breeding season (spring), the concentration was high. The authors thought that environmental cues stimulated the reproductive system in the frog by an increase in LHRH production and secretion.

In lower vertebrates there are three main stages in the formation and development of eggs. Oogonia divide and transform into oocytes in the first stage. Growth phases of the oocytes occur in the second and third stages. The first growth phase (FGP) does not depend on gonadotrophin, but the second growth phase (SGP) is gonadotrophin dependent. "The gonadotrophin-dependent growth phase is characterized by uptake of yolk precursors from the blood and their deposition in the oocytes. Hence, the phase is also known as the vitellogenic growth phase" (Jorgensen, 1973). In annually breeding anurans, it takes three years to produce mature eggs, approximately one year for each of the three different stages. Atresia is insignificant in FGP. It occurs mainly in later phases of vitellogenic growth or in eggs that did not ovulate during the spawning season. Jorgensen (1973) studied the pattern of recruitment of oocytes to second growth phase in normal toads, and in hypophysectomized toads, *Bufo bufo bufo* (L.), treated with gonadotrophin (HCG). Sensitivity toward gonadotrophin increases with the size reached by the oocytes during the FGP. "Recruitment to SGP is not immediately followed by oogenesis and first growth to restore number and size frequency distribution within the population of FGP oocytes". Jorgensen (1974) concluded that secretion of estradiol-17 $\beta$ , or other estrogens from the ovary does not seem to play an essential role in the mechanisms that control gonadotrophin secretion during the period of vitellogenic growth in the toad ovary. In the postspawning period the rate of gonadotrophin secretion is directed by an autonomous activity of the hypothalamic gonadotrophic centre.



In the female lizard *Sceloporus cyanogenys* Callard *et al.*(1972) found that the normal rate of gonadotrophin secretion is not dependent upon negative feedback by ovarian estrogens as an essential part of the regulatory mechanisms. "It is thus indicated that the hypothalamo-hypophysial gonadotrophic system in female nonmammalian vertebrates may generally be more sensitive to stimuli classified as stressors than to changes in levels in circulating estrogen."—(Jorgensen, 1974).

Jorgensen(1973) studied the mechanisms regulating ovarian function in amphibians (toads). Temperature seems to be the most important synchronizing factor but an inherent rhythm in the activity of gonadotrophic centre cannot be ruled out.

Jorgensen(1974) suggested that the mechanisms that regulate influx of first growth phase oocytes to the gonadotrophin dependent growth phase are mainly to be found within the ovary itself.

Growth of oviduct depends upon the growth of ovaries. Rapid decline in weight of the oviducts occurring during the breeding period has been noted by Jorgensen and Vijayakumar(1970) only in spawned toads and it may be due to the depletion of the secretory granules stored in the gland cells of the oviducts. Mechanical stimulus exerted by the passing eggs leads to the release of contents of the gland cells.

Vijayakumar, Jorgensen and Kjaer(1971) suggested that specific ovarian factor(s) secreted to the blood may participate in regulating ovarian function, specially recruitment of oocytes to vitellogenic growth phase, for instance, by regulating(decreasing) the sensitivity of the ovary toward gonadotrophin.

Regarding the function of ectopically transplanted pars distalis, Jorgensen (1970) did not find secretion of measurable amounts of ACTH. TSH secretion is strongly reduced, as judged by the low rate of  $^{131}\text{I}$  uptake by the thyroid gland. Ectopic pars distalis secreted FSH which could maintain a fairly normal spermatogenesis, but Interstitial Cell-Stimulating Hormone(ICSH) secretion could not maintain the thumb pads(a secondary sex character). No secondary sex characters are found in the female toad and only ovarian growth was evaluated in females. Seasonal variation has been noted regarding the dependence of gonadotrophic function upon hypothalamic contact. During the months following the breeding season, little difference could be observed between ovarian growth in females with the pars distalis transplanted to an eye muscle and in females with pars distalis regrafted under the median eminence. Uncontrolled release of MSH was found in denervated pars intermedia.

Transection of the ventral brain stem caudal to the optic chiasma showed normal gonadotrophic and thyrotrophic function, but the corticotrophic function was abolished. MSH secretion was high. When the level of transection was



anterior to the optic chiasma, all hypophysial functions studied were more or less normal except the thyrotrophic function. It was significantly enhanced. Neurons originating in the posterior hypothalamus between the hypophysis and optic chiasma can maintain normal thyrotrophic and gonadotrophic functions. For normal corticotrophic function the hypothalamo-hypophysial complex should include the region of the optic chiasma. Rates of  $^{131}\text{I}$  uptake by the thyroids increased after transection of the hypothalamus in front of the optic chiasma, but the uptake was low after ectopic transplantation of pars distalis. So the control of TSH secretion involves inhibitory as well as stimulatory nervous components. However, in the frog *Rana temporaria* thyroid  $^{131}\text{I}$  uptake was high after transections either anterior or posterior to the optic chiasma and no significant effect could be observed. The transplanted pars distalis in the frog showed pronounced autonomous thyrotrophic activity.

While discussing the neuroendocrine control of ovarian cycle Jorgensen(1970) concluded that the presence of a normal population of growing oocytes in an ovary prevents further recruitment to the population. vitellogenesis in other oocytes is not prevented by the population of growing or full-grown oocytes by a local inhibitory mechanism. "It seems reasonable to suggest that recruitment to the vitellogenic growth phase may be regulated by a feedback mechanism in which secretion of hormone from the growing or full-sized follicles controls the gonadotrophic activity of the hypophysis.....A self-regulatory mechanism for maintenance of a specific number of gonadotrophin-requiring oocytes in the ovaries might thus operate even at constant autonomous levels of gonadotrophin secretion". Rapid replenishment of the lost population of oocytes does not occur in toads with the pars distalis transplanted to an eye muscle. The rapid rate of growth of oocytes in autumn and winter appears to depend on central nervous stimulation of gonadotrophin secretion from the pars distalis. Slight stimulation of gonadotrophin secretion was found in spring. Minimal difference in ovarian development in toads could be observed with pars distalis regrafted under the median eminence or autotransplanted to an eye muscle. Jorgensen(1970) said, "It is therefore suggested that one principal factor in the regulation of the annual ovarian cycle in the toad is an annual rhythm in the activity of gonadotrophin-controlling structures in the brain. These structures may be located in the posterior hypothalamus".

It is not known whether apart from increased temperature and rainfalls, prolactin plays a part in the migration of toads from hibernation quarters to breeding ponds in spring. In urodeles prolactin plays a role in water-drive. Contact of the skin with water induces ovulation in the toad. Claspings of the male is less important. Depressing effect of starvation on the hypophysial-ovarian function can be counteracted to certain extent by central nervous stimulation (Jorgensen,1968). "Period of hibernation plays an important role in maintaining and synchronizing the normal annual ovarian cycle". "The function of the brain-hypophysis-ovary complex disintegrates easily".



*Control of ACTH secretion*

Jorgensen(1968, 1970, 1976) and Holmes and Ball(1974) reviewed this subject. *Bufo*, *Triturus*, and *Ambystoma* die within some weeks after hypophysectomy but hypophysectomized *Xenopus laevis* may survive for several months (Jorgensen,1968). Treatment with ACTH restores good health in hypophysectomized *Bufo bufo*.

Roy(1960) found that isolated toad (*Bufo melanostictus*) hypophysis does not secrete significant amounts of ACTH. No corticosteroids could be found in the blood of hypophysectomized toads or of toads bearing ectopic autografts of the hypophysis. Measurable amounts were present in the blood of toads in which the hypophysis had been regrafted under the median eminence.

In the toad moulting becomes abnormal after hypophysectomy and normal moulting can be restored by injection of small amounts of ACTH (Jorgensen and Larsen,1963). Little or no difference could be found between hypophysectomized toads and toads with an ectopically transplanted pars distalis with respect to either survival or abnormal moulting. This indicates that ACTH is not secreted from the ectopic pars distalis (Jacobsohn and Jorgensen,1956). Jorgensen (1968) found that sectioning of the hypothalamus just in front of and behind the optic chiasma practically always reduced ACTH secretion from the hypophysis. In most of the toads ACTH secretion was less reduced than in toads with ectopically transplanted pars distalis. These evidences prove that brain structures responsible for the maintenance of corticotrophic function are situated anterior to the middle hypothalamus.

Jorgensen(1970) located the ACTH-controlling structures in the middle hypothalamus.

Dierickx and Goossens(1970) observed normal adrenocorticotrophic activity of the pars distalis of the hypophysis of *Rana temporaria* when neural connections with the brain was intact. After complete neural disconnection, the pars distalis of the hypophysis maintains a significant residual adrenocorticotrophic activity.

Roy(1960) and Buchmann *et al.*(1972) observed undetectable levels of plasma corticosteroids in *Bufo* spp. after hypophysectomy. Similar result was achieved by both the groups of investigators in toads with ectopically grafted pars distalis.

Urodeles die after hypophysectomy with reduction in the ability to regenerate lost parts of the body.

Buchmann *et al.*(1972) found that ACTH controlling neurons originate within a fairly restricted hypothalamic area at the level of the optic chiasma.



Roy(1957) studied the hypophysiportal circulation, hypothalamus, pituitary and adrenal of the toad (*Bufo melanostictus*) and changes in them after fracture and other stresses. Importance of the hypophysiportal circulation was stressed and he stated, "It is also to be kept in mind that reunion of the severed portion (of the stalk) by vascular granulation tissue is a possibility and actually it did happen in some of the preparations after stalk section". The neurosecretory substances were noted in the median eminence from where they were carried to the pars distalis by hypophysiportal vessels. Loss of neurosecretory substance was noted in the hypothalamic nuclei and neurohypophysis after stress and replenishment of the substance occurred at varied intervals after one hour.

Roy(1970) conducted grafting experiments of the pituitary into the hypophysiotrophic area. Plasma corticosteroids could not be measured in hypophysectomized toads (*Bufo melanostictus*). It could be measured (trace) in hypophysectomized animals with grafts into the hypophysiotrophic area(HIA). The level was normal in hypophysectomized animals with grafts in HIA and when the grafts were maintained by vascularity from the hypophysiportal vessels.

Jorgensen(1976) reviewed the works of Roy(1969-71). Roy electrically stimulated or lesioned different parts of the toad (*Bufo melanostictus*) brain and then measured plasma corticosteroids. Electrical stimulation raised plasma corticosteroids mostly. Highest values were obtained after stimulation in the area of the preoptic nucleus, the ventral hypothalamus, or the median eminence. The mean values obtained were 14.1, 12.8 and 15.5 $\mu$ g/100ml plasma respectively. These values were significantly higher than in the sham stimulated control groups where plasma corticosteroids averaged about 6 $\mu$ g/100ml, against 2.5 $\mu$ g in the unoperated controls. Stimulation of the primordium hippocampi significantly reduced plasma corticosteroids to a level of 3.3 $\mu$ g/100ml. The lesion experiments corroborated those of stimulation experiments. Lesions in the preoptic area and specially, in the ventral hypothalamus and median eminence reduced corticosteroid levels in the plasma, whereas lesions of the primordium hippocampi permanently increased corticosteroid levels, the level being 10.3 $\mu$ g/100ml two weeks after the operation. These results agree with those of Buchmann and coworkers(1972) on *Bufo bufo* in suggesting that ACTH controlling neurons arise in the anterior hypothalamus. These neurons are controlled by extrahypothalamic nerve tracts and inhibitory tracts arise in the dorsal part of the fore-brain.

The chemical nature of amphibian CRF is not definitely known. Effect of CRF can also be achieved by various pars nervosa hormones, both homologous (vasotocin) and heterologous (arginine and lysine vasopressin) (Jorgensen and Larsen, 1960, 1963—in toad) (Dupont and Peltier, 1970—in frog). The vasopressins were more active.



Interrenal activity declines in some crested newts bearing a pituitary autograft (Mazzi, 1970). 4 months after operation (unlike *Pleurodeles*—Pasteels, 1960), the cells are much smaller than normal and show shrunken nuclei. Schotte and Tallon (1960) and Dent (1967) observed that in *Triturus viridescens* bearing a pituitary autograft, limb regeneration capacity was maintained. This may be due to increased secretion of prolactin by the grafted pituitary rather than to normal ACTH production (Mazzi, 1970).

Leboulenger *et al.* (1979) conducted seasonal study of the interrenal function of the European green frog *in vivo* and *in vitro*. Seasonal fluctuations have been noted in corticosterone production. As the fluctuations cannot be due to variations of ambient temperature, their *in vitro* results support the view "that plasma corticosterone rhythms are due, at least in part, to seasonal variations of interrenal sensitivity to ACTH".

Immobilization stress was utilized in frogs by Jurani *et al.* (1973) immediately after the animals were caught. Frogs (*Rana esculenta*) were hung by a hind leg for 5, 10, 30 and 60 min. Plasma corticosterone concentrations increased proportionately during the period of observation from 1.6  $\mu\text{g}/100\text{ ml}$  in the controls to 2.2  $\mu\text{g}/100\text{ ml}$  at 5 min, 3.8  $\mu\text{g}/100\text{ ml}$  at 10 min, 5.2  $\mu\text{g}/100\text{ ml}$  at 30 min, and 5.6  $\mu\text{g}/100\text{ ml}$  at 60 min. Noradrenaline content of the adrenorenal homogenates also increased after immobilization. It increased further after prolonged immobilization upto 60 min. No change could be detected in corticosterone and adrenaline content of the adrenorenal homogenates throughout the period of immobilization.

Resting plasma corticosterone level did not change below 20°C, but significant rise could be observed above 20°C.

Rise in plasma corticosterone concentration did not occur at 1°C after ACTH. At higher temperatures (upto 30°C) a significant correlation could be detected between plasma corticosterone levels after ACTH administration and environmental temperature. Between 30°C and 40°C there was no further increase in plasma corticosterone level.

Hanke (1978) reviewed the unpublished observations of König regarding blood concentration level of corticosterone in *R. temporaria* at 30°C for 10 days. It increased from 3.8 to 5.8  $\mu\text{g}/100\text{ ml}$ . Elevated levels could be detected one and two days after the experiment was started. The concentration dropped to less than normal levels by the third day. Increase was noted again from days four to nine. Thereafter the corticosteroid concentrations fell again and most of the frogs died. Relationship between catecholamines and interrenal activity was also examined in *Rana temporaria*. Interrenal activity is primarily or secondarily stimulated by the release of catecholamine from the chromaffin tissue.



Hypothalamic catecholamine content in *Xenopus laevis* and *Tilapia mossambica* during hyperosmotic adaptation was studied by Abo Hegab *et al.* (1980). Changes in adrenaline and dopamine content are possibly involved in the release of ACTH, or prolactin (from Ball and Batten, 1980).

Johnston *et al.* (1967) observed increase in plasma corticosterone in *R. catesbeiana* after administration of mammalian ACTH. Plasma aldosterone level increased after acute bleeding; but there was no change in corticosterone level. Corticosterone and aldosterone output increased after ACTH and production of both steroids diminished after hypophysectomy.

Dehydration leads to decrease in serum corticosterone level in *R. pipiens* (Jungreis *et al.*, 1970).

Participation of hypothalamus in the response of interrenal tissue to stress in the frog *Rana ridibunda* could be observed by Turekova and Jurani (1978).

*In vitro* production of corticosteroids was found to be depressed in *R. catesbeiana* after daily injection of corticosterone or aldosterone for 14 days (Piper and deRoos, 1967).

Braverman *et al.* (1973) studied the effect of sodium depletion and postcaval vein constriction on steroid secretion in the bull frog (*R. catesbeiana*). These experiments have been conducted with an idea to find evidence on the physiological role of the renin-angiotensin system and ACTH in the control of both deoxycorticosterone (DOC) and aldosterone secretion. Depletion of sodium resulted in an increase in the secretion of aldosterone, corticosterone, and DOC. After postcaval vein constriction, a similar pattern of response could be detected. Dexamethasone inhibited ACTH secretion in the bullfrogs and in such animals aldosterone secretion increased significantly after sodium depletion or constriction of postcaval veins. No significant increase could however be observed in DOC and corticosterone output. ACTH plays an important role in adrenal response to sodium depletion and caval constriction. ACTH controls DOC secretion in these two experimental conditions. A decrease in plasma sodium is responsible for the increase in aldosterone secretion when the function of the anterior pituitary was depressed. Analysis of the steroids was performed in postcaval vein plasma by the double-isotope derivative method.

Stress response was studied by Laub *et al.* (1975) in frogs (*Rana pipiens*). The animals were hung by one hind leg for 30 minutes before decapitation. An increase in the level of plasma corticosterone was observed. This increase could however be prevented by implants of corticosterone or aldosterone in the brain.

Implantation of corticosterone, aldosterone or betamethasone in the antero-ventral hypothalamus or in the median eminence depressed blood corticosterone



level. Cholesterol implants in the same areas had no action. Implantation of the hormones outside the anterior part of the basal hypothalamus was also ineffective.

Hanke(1978) suggested that ACTH-like activity in the brain tissue of amphibians may be due to MSH as ACTH molecule chemically resembles MSH. Experiments on *Xenopus* were conducted to understand the nature of ACTH-like activity in different parts of the brain. In hypophysectomized *Xenopus* tadpoles different parts of the brain and the pituitary were transplanted to the kidneys. The steroid dehydrogenase activity was semi-quantitatively measured after 4-7 days. Significant increase in the level was obtained after grafting of pars distalis, hypothalamus or basal mesencephalon adopted from normal juvenile *Xenopus laevis*. When the grafts were taken from hypophysectomized juveniles (hypophysectomized for 2, 14 or 90 days) only hypothalamic tissue increased the enzyme activity. Grafts of basal mesencephalon could not increase the enzyme activity. Hanke(1978) concluded that: the ACTH-like activity of the hypothalamus is not dependent on the pituitary. ACTH-like activity of the basal mesencephalon depends on the intact pituitary and so may be due to bound-ACTH released from the pituitary. Cerebral tissue has no ACTH-like activity. "There is no correlation between the ACTH-like activity of the hypothalamus and the basal mesencephalon when donors are given metyrapone and dexamethasone". "Biogenic amines in the brain may donate primary or secondary ACTH-like activity and indirectly affect interrenal activity".

Gastrin immunoreactive sites (nucleus infundibularis ventralis of the pars ventralis of the tuber cinereum, anterior preoptic area, and external zone of the median eminence) correspond to the steroid hormone uptake sites in the brain of *Xenopus laevis* (Doerr-Schott *et al.*,1980) (from Ball and Batten, 1980).

Substance P-like immunoreactive material was found in the external zone of the median eminence, and in occasional cell bodies located in the posterior hypothalamus and in the preoptic area (parvocellular neurons) of *Xenopus* and *Triturus* (Gaudino *et al.*,1980). Positive fibres were noted in the posterior hypothalamus and in the preoptic area (from Ball and Batten, 1980).

Pelletier and Desy(1979) investigated the localization of ACTH in the human hypothalamus. Neuronal cell bodies situated exclusively in the arcuate nucleus of the human hypothalamus contain ACTH. Extensive distribution of ACTH-containing fibres has been noted in the hypothalamus with greatest density in the periventricular nucleus. Concentration of ACTH fibres could not be observed in the neurovascular zone of the pituitary stalk. Pelletier and Leclerc(1979) studied immunohistochemical localization of adrenocorticotrophin in the rat brain. Maximum ACTH immunoreactivity in the fibres could be



observed in N. proprius of the stria terminalis, N. periventricularis, N. paraventricularis hypothalamus, N. paraventricularis thalamus, and periaqueductal grey and N. parabrachialis in mesencephalon. Only cell bodies in arcuate nucleus and periarculate area showed maximum ACTH immunoreactivity.  $\beta$ -LPH and ACTH are situated in the same cells in the anterior and intermediate lobes of the pituitary gland. Cells of intermediate lobe also contain  $\alpha$ -MSH. Vesicles containing ACTH are 60-80 nm in diameter, whereas those positive for  $\alpha$ -MSH are 40-70 nm in diameter. The authors therefore suggested that these peptides are produced by different types of neurons. ACTH of nonpituitary origin could probably be considered as a neurotransmitter of still undefined function.

#### *Pars intermedia controlling structures*

Inhibitory neurons for MSH control originates at the level of the optic chiasma. Enemar *et al.* (1967) transected the hypothalamus 0.1 to 0.5 mm caudal to the optic chiasma of *Bufo arenarum*. They found that the animal turned completely dark. An intermediate colour was adopted when the level of section was immediately in front of the optic chiasma. Similar experiments were conducted by Jorgensen (1968). In the unoperated or sham-operated control, the melanin in the melanophores was concentrated in *Bufo bufo* on a white background and dispersed in toads on a black background.

In toads with hypothalamic lesions the degree of melanin dispersion did not vary with the background. Lesion caudal to the optic chiasma produced more dark toads than the group with rostral lesions. Effect of rostral lesions was due to blinding of the toads. Caudal lesions also made them blind due to injury to the optic tracts. These experiments indicate that hypothalamic inhibitory neurons take their origin at the level of and perhaps a little caudal to the optic chiasma. These neurons inhibit MSH secretion in the pars intermedia.

Jorgensen and Larsen (1960) concluded that pars intermedia function in *Xenopus* and *Bufo* appeared to be controlled by secretory nerves and inhibitory nerves. Denervation of the pars intermedia led to darkening of the toads due to uncontrolled release of MSH and this causes dispersion of melanin granules within the melanophores. After several months some denervated toads kept on a white illuminated background resumed the ability to concentrate the melanin granules. This may be due to regeneration of the inhibitory innervation of the gland (Jorgensen and Larsen, 1963).

Etkin (1962) also showed by lesion experiments that amphibian pars intermedia is under hypothalamic inhibitory control. This was also confirmed by Iturriza (1965).



Rodriguez, La Pointe and Dellmann(1971) thought that in the pars intermedia there are at least two types of nerve fibres : neurosecretory and nonneurosecretory. By fluorescence and ultrastructural studies the non-neurosecretory fibres appear to be aminergic (Enemar, Falck and Iturriza, 1967; Bjorklund, 1968; Saland, 1968; Naki and Gorbman, 1969). The inhibitory control of pars intermedia is by these nerves (Iturriza, 1965, 1969; Enemar and Falck, 1965; Goos, 1969).

Rodriguez, La Pointe and Dellmann(1971) studied intermediate lobes of frogs (*R. pipiens*) and toads (*Bufo arenarum* H). In the intermediate lobes of the toads AF+fibres could be traced as is seen in the neural lobe. Their situation in the intermediate lobe is close to the neural lobe. Plenty of fibres can be detected throughout the gland which are stained by silver techniques. Ultrastructurally, the fibres are of two types. Type B fibres with endings containing clear vesicles (40nm) and granules (60-80nm in diameter) are met with. These inclusions are also seen in dilatations in preterminal portions of the fibres. There are type A fibres containing 40nm vesicles and granules from 130 to 150nm in diameter. The type B fibres are found in all parts of the gland. Type A fibres are found only in the region of the pars intermedia which is nearest to the neural lobe.

Twentyfour hours after transection of the infundibulum in frogs there was no change in both the types of fibres. Between six to nine days after transection no nerve fibres in pars intermedia could be found. Marked changes in the secretory cells occurred. Six to twelve hours after the operation the dense secretory granules looked larger and paler. The secretory cells showed only a few secretory granules restricted to the Golgi area and profound proliferation of the rough endoplasmic reticulum, 6 to 9 days after transection.

Marked darkening of the skin of transected frogs took place between 2 to 4 hours after the operation.

Rodriguez *et al.*(1971) concluded that there are two types of fibres in the amphibian intermedia. AF+fibres are type A fibres and silver stained fibres are type B fibres. They said, "In the frog, the disappearance of the fibres after transection together with the striking hypertrophy of the RER and the darkening of the animals suggest that the inhibitory control of pars intermedia is exerted by fibres coming from the infundibulum and that most of them reach the secretory cells of the p. intermedia." In amphibians and mammals the inhibitory control of p.intermedia is probably exerted by a neural and a neurovascular mechanism.

Jorgensen and Vijayakumar(1971) obtained results from lesion experiments of the hypothalamus of *Bufo bufo* to suggest that for the normal melanophore response to a black illuminated background, neurons taking their origin in the



anterior hypothalamus of the toad are necessary. The neurons may act by stimulating the rate of secretion of MSH from the pars intermedia. They may act indirectly by inhibiting the inhibitory nerves or there may be innervation of the pars intermedia directly.

Ultrastructurally two types of innervation have been met with: type A and type B. Doerr-Schott and Follenius(1970) found nerves without granules innervating the pars intermedia in *Rana esculenta*. These fibres are thought to be cholinergic.

In anaesthetized, curarized frogs (*Rana pipiens*) Oshima and Gorbman(1969) recorded electrical activity in single nerve fibres in the pars intermedia. Two types of neurons firing spontaneously were found by them. One type of neuron was indifferent to changes in illumination. The other type was inhibited by light that appeared to act via the pineal organ and not through the eyes. Mediation of the background response by the two types of neurons via the eyes remained uncertain. The first type corresponded to type B aminergic fibres. These inhibit MSH secretion and travels laterally in the infundibular floor. The second type probably stimulates MSH release and the fiber travels medially in the infundibular floor.

Davis and Hadley(1979) studied the possible influence of the epithalamic area on MSH release in *Rana berlandieri forrei* (*Rana pipiens*, *sensu lato*). There was a reversible darkening of skin melanophores after electrical stimulation of a specific area on the diencephalic roof. The latency period between stimulation and starting of melanophore dispersion was from 1-3 minutes. Hypophysectomy prevented the response. The pars intermedia MSH secretion is thus regulated by "a neural, humoral, or integrated neuroendocrine pathway". The pathway apparently develops "from the diencephalon, in proximity to the pineal-subcommissural complex".

LHRH stimulated MSH release by the bullfrog pars intermedia *in vitro* (Dickhoff, 1974). Vaudry *et al.*(1977) observed that TRH could stimulate MSH secretion by the pars intermedia of a frog.

Electrophoretic and chromatographic separation and identification of the intraglandular, acid activatable, and secreted forms of melanotrophic peptides were done by Dickhoff and Nicoll(1979) from neurointermediate lobe of the American bullfrog, *Rana catesbeiana*. Five forms of MSH could be detected on acrylamide gels. Their results indicated that the pars intermedia of the bullfrog contains multiple forms of MSH but predominantly only one form is secreted which resembles mammalian  $\beta$ -MSH. A high molecular weight form is contained in the gland which is converted to smaller forms by acid treatment.



$\alpha$ -MSH-like peptide is contained in the frog brain extracts (Vaudry *et al.*, 1978). Enzymatic mechanisms exist in rat plasma, brain, liver and frog brain which can degrade the  $\alpha$ -MSH.

The pars intermedia of the amphibian hypophysis contains peptidergic, catecholaminergic and possibly cholinergic nerve fibres (Dierickx and Vandesande, 1976). Dierickx and Vandesande (1978) concluded that the *mesotocinergic* nerve fibres of the amphibian (*Rana temporaria*, *R. esculenta*, and *Bufo bufo*) pars intermedia are axons of neurosecretory cells situated in the hypothalamic magnocellular preoptic nuclei. Mesotocinergic and vasotocinergic neurons have been found to be separate in the amphibian hypothalamic magnocellular neurosecretory nuclei. The authors infer that the *mesotocinergic* nerve fibres of the pars intermedia must contain mesotocin and/or parts of the mesotocin molecule. The authors further stated, "As described, our light-microscopic immunocytochemical observations suggest that the amphibian pars intermedia contains no, or very few, vasotocinergic fibres. By contrast, according to our preliminary electron-microscopic studies, the amphibian pars intermedia would also contain separate vasotocinergic fibres distributed throughout the whole gland. The reason for the discrepancy between our light-and electron-microscopic results is being investigated".



*Hypophysiotrophic area and deafferentation of the medial basal hypothalamus*

The importance of the hypophysiotrophic area in the control of the anterior pituitary of the amphibians by grafting experiments has been mentioned before. In the following pages its importance in the rat and rabbit has been described.

The hypothalamic region which can maintain the basophils of the implanted pituitary has been termed as the *hypophysiotrophic area* (HTA) by Halasz *et al.* (1962). Anteriorly it extends in the level of the optic chiasma from the ventral surface up to the paraventricular nuclei or even slightly above. Posteriorly the dorsal border gradually comes down ventrally and in the anterior mamillary level it is limited to a narrow zone around the inframamillary recess of the third ventricle. Medio-laterally the area extends  $\frac{1}{2}$  mm or slightly more, from the midline.

Three distinct cytoarchitectonic subdivisions of the medial hypothalamus are situated in the HTA : (i) the whole of *arcuate nucleus*; (ii) the medial parvicellular part of the *retrochiasmatic area* (the magnocellular lateral part is situated outside the area); (iii) only a small portion of the ventral (rostral) part of the *anterior periventricular nucleus*.

This region can preserve pituitary basophil cells and pituitary grafts in this area and can maintain trophic hormone secretion as is found with pituitaries of normal rats. Secretion of gonadotrophic hormone, ACTH, TSH and growth hormone by the transplanted pituitary is maintained if the pituitary graft is situated only in the hypophysiotrophic area (Szentagothai *et al.* 1968). The nerve cells of the medial basal hypothalamus can produce the *hypophysiotrophic factors* and they are carried by the tubero-infundibular tract to the median eminence. These factors enter the portal circulation to reach the anterior lobe of the pituitary. Compensatory hypertrophy of the remaining adrenal gland after unilateral adrenalectomy occurs in animals with pituitary transplants in the hypophysiotrophic area only when the graft is closely connected with the median eminence. This is not so for ovarian compensatory hypertrophy. For an appreciable TSH response to methylthiouracil treatment, pituitary graft must come into direct contact with the median eminence. "The *trophic* factors essential for the maintenance of gonadotrophic hormone secretion would be present in active form and sufficient amount in the nerve cells of the hypophysiotrophic area, whereas those for ACTH and TSH only at the nerve endings."

Halasz knife was utilised by the authors to produce (a) *complete deafferentation* (hypophysiotrophic area was completely cut around, leaving the area in contact with the pituitary by uninterrupted pituitary stalk), (b) *incomplete deafferentation* (where the dorsal and posterior connections of the hypophysiotrophic area were bilaterally interrupted leaving the medial basal hypothalamus



in contact with the anterior hypothalamus) and (c) *frontal cut* (where the anterior connections of the hypophysiotrophic area were divided leaving the other connections intact).

Though the HTA can function independently, still it is insufficient by itself to maintain the hormone secretion of the anterior lobe at a normal level. After neural isolation of HTA, ovulation and ovarian compensatory hypertrophy are blocked, and normal pituitary TSH response to propylthiouracil does not occur. When the anterior afferents to the HTA are interrupted by frontal cut, the diurnal rhythm of ACTH secretion is interfered, but there is alteration of the rhythm when all other neural connections are divided.

The first level of control of the pituitary is by the hypophysiotrophic area through its hypophysiotrophic substances. The Releasing Factors system is a *parvicellular neurosecretory* or *tubero-infundibular* neuron system. The second level of control is by the nervous structures situated outside the HTA. These nervous elements act through the HTA by regulating the synthesis and release of hypophysiotrophic substances produced by this area. "Everything outside the confines of HTA and concerned in the nervous control of anterior pituitary functions is the Release Regulating System (RRS)." In the median hypothalamus they are ventromedial, anterior and premamillary nuclei. Extradiencephalic regions of RRS are the preoptic area, the septum, the amygdala, the habenular region and others.

Makara *et al.* (1980) reevaluated the pituitary-adrenal response to ether in rats with various cuts around the medial basal hypothalamus. Previously it was thought that corticotrophin-releasing factor (CRF) is produced by nerve cells situated in the medial basal hypothalamus (MBH). This view is now in doubt because (i) in rats with complete deafferentation of the MBH, no ACTH release could be achieved by electrical stimulation of the isolated tissue and (ii) CRF content of the stalk median eminence (SME) proper dropped to an undetectable level. The present results support the hypothesis that CRF-containing fibres enter the MBH from outside and that most of these fibres run through the lateral retrochiasmatic area (RCAL) on their way towards the neurohaemal regions of the infundibulum. A small but significant rise in plasma corticosterone in response to ether inhalation was found in rats with a *small* MBH island (shorter than the rostrocaudal extent of the median eminence). Some CRF-containing fibres might have reached the regenerated portal blood vessels. However, large piece of isolated MBH (diameter of 4 mm) did not show such response. Danilova and Polenov (1977) observed *Gomori-positive* neurosecretory sprouting in the MBH after deafferentation of the same in the rat. Makara *et al.* (1980) had similar findings. Such neoformation of neurohumoral link is more possible with small hypothalamic islands and when the animals survive for a long period after surgery. Vascular connections may form between two sides of the hypothalamic



scar. In all rats with *anterolateral cut* there was atrophy of the neural lobe with disappearance of Gomori-positive neurosecretory material.

In the fourth Geoffrey Harris Memorial Lecture, Flerko (June, 30, 1979 and published in 1980) reviewed their works and those of others on *The hypophysial portal circulation today*. Flerko and colleagues did not find LHRH synthesizing neurons in the HTA of the rat by immunohistological techniques between 1974 and 1979. They described immunoreactive LHRH nerve fibres and terminals in the median eminence of the rat. This delicate fibre system could be traced back to the preoptic-suprachiasmatic region and arcuate nuclei area but no LHRH immuno-positive nerve cells could be found in those areas. Subsequently occasional LHRH-positive nerve cells could be detected in the preoptic-suprachiasmatic and medial prechiasmatic areas of intact, adult, female rats killed at the end of diestrus or in proestrus. This may be due to low LHRH content of the nerve cells which is below the threshold of their immunological technique. They tried to increase the neurohormone concentration in LHRH containing cell bodies by experimental manipulations which interfere with the axoplasmic transport or release of the neurohormone. Nembutal was injected into adult female rats before and/or during the so-called *critical period* or a frontal cut was made with Halasz knife. LHRH + cell bodies were found to be scattered in the preoptic-suprachiasmatic area near the organum vasculosum of the lamina terminalis (OVLT). Arcuate nuclei did not contain nerve cells producing LHRH. This fact was proved by their observations in rats with *Completely isolated MBH*. After two weeks of surgery, a very few LHRH + fibres entered the hypothalamic island in the most superficial layer of the median eminence which might have escaped the knife cut. A fair number of LHRH + fibres and terminals could be found in the *frontally deafferented* median eminence (frontal cut behind the optic chiasma) including the area of arcuate nuclei. No LHRH + cell bodies were found however. It proves that axons of LHRH + nerve cells in the preoptic-suprachiasmatic area situated in front of the frontal cut proceed towards the middle part of the MBH above the plane of surgery. These fibres pass through the arcuate nuclei and reach the superficial layer of the median eminence. In their course before the arcuate nuclei they are situated in the periventricular gray matter on both sides of the third ventricle. The terminals of the axons end on or near the capillary loops of the hypophysial portal vessels. Axons originating from the most rostrally located LHRH + nerve cells near the OVLT end on the capillaries of this organ. These findings are at variance from those of Naik(1975) and Hoffman *et al.*(1978) who found LHRH + perikarya in the arcuate, ventromedial and paraventricular nuclei of the rat brain. LHRH + perikarya are situated in the preoptic-suprachiasmatic area of the rat, guinea pig and cat. About 40% of such cells are situated in the tuberal-premamillary region of the dog. In primates and humans majority of LHRH + cells can be found in the tuberal-premamillary area. In the dog, cat and primates such cells can be easily detected. In the rat and guinea pig detection of these



cells requires stimulation of synthesis of LHRH and/or blockage of the axoplasmic transport or release. Vigh *et al.*(1978) used sulpiride or reserpine. Sulpiride blocks ovulation and axoplasmic transport or release of LHRH is inhibited either by sulpiride or reserpine. Reserpine depletes norepinephrine which is stimulant for LHRH release. Therefore in absence of norepinephrine there will be increase in LHRH content of the cells in reserpine-treated rats.

After treatment with sulpiride or reserpine, LHRH-containing nerve fibres and terminals were plenty in number in the median eminence and OVLT. LHRH + nerve fibres were also found in the anterior periventricular gray matter of the third ventricle of female rats. Extrahypothalamic regions of rabbits also contained LHRH + nerve fibres.

Hypophysiotrophic area (HTA) did not contain LHRH + cells. LH and FSH were detected in the anterior pituitary homografts placed in the HTA by peroxidase-labeled antibody method. Proper vascularization of the transplants is necessary for the presence of the hormone-containing cells in the homografts *irrespective of their location*. The hormone content in the *hypertrophic gonadotrophs* is low indicating a release of the hormone from these cells. The hypertrophic gonadotrophs are found *only in the grafts situated in the HTA*. Inactive (small) gonadotrophs are found in grafts situated outside the HTA or grafts under the renal capsule. These cells are in *storing stage*. Diffusion and formation of new LHRH terminals on or near the pituitary grafts may not be the source of LHRH for the hypophysiotrophic effect of HTA. This effect can however, be considered through circulation via the subependymal arteries and ascending branches of the three hypophysial arteries (retrograde circulation to the HTA). Hypophysiotrophic neurohormones are thus supplied to the pituitary graft in the HTA. There are no hypertrophic and actively secreting LH cells in pituitary transplants situated in the HTA *when the median eminence of these rats is removed completely*. Flerko(1980) said, "The original concept of Halasz *et al.*(1962) must be modified by assuming that the vascular link between capillaries of the median eminence and the HTA is the decisive factor in the hypophysiotrophic effect of the HTA."

Changes in the direction of blood flow have been observed in the lizards by Szentagothai and in mice by Worthington Jr. Flerko *therefore thinks that vasomotor influences may lead to fundamental changes in different functional states*.

Setalo *et al.*(1978) suggested that LHRH cells do not take part in the storage of the hormone in animals having a relatively short estrous cycle. In the rabbit release of an ovulatory dose of LHRH takes place, only in response to genital



stimuli. Relatively large amount of LHRH is expected to be present in absence of such stimuli. The authors studied virgin rabbits and rabbits mated within 1-48 hours before fixation. In virgin female rabbits cells containing LHRH were found both in the suprachiasmatic and tuberal regions. LHRH positive fibres could easily be found in the tuberoinfundibular and praeoptico-infundibular tract. In the periventricular area, just under the ependymal layer, a plexus of LHRH fibres could be found, which carried axons probably from the preoptic-suprachiasmatic area to the median eminence. LHRH axons were also located in the mamillary nuclei and posterior to them, at the mesencephalic end of the tractus habenuleduncularis, in the medial habenular nucleus, around the emerging fibres of the habenuleduncular tract, in the stria terminalis, in the stria medullaris thalami, and near the diagonal band of Broca. LHRH cells could be found among the LHRH axons near the diagonal band of Broca. These axons proceeded towards the dorsal part of the septum pellucidum where they mixed with other fibres. The other end of this bundle was traced by the authors along the olfactory tract close to the ventral surface upto the olfactory bulb. Shortly after copulation, LHRH content of the brain diminished. 1-48 hrs. after copulation, LHRH axons were only noted in the tuberal and habenular areas.

Danilova(1978) studied classical (Gomori-positive) neurosecretory system in the rat after isolation of the medial basal hypothalamus (MBH) and adrenalectomy. Regeneration of Gomori positive neurosecretory fibres with formation of new axovascular contacts occurs after deafferentation (DA) of the MBH. The amount of Gomori + material is higher in the hypothalami of DA + adrenalectomized (AE) rats when compared to that in the hypothalami of rats with DA or only AE. After complete isolation of MBH there are no CRF-granules in the external median eminence in the rats 2-3 weeks after adrenalectomy. *The author suggests that Gomori + cells of the anterior commissural nucleus synthesize CRF.*

#### *Tanycyte ependyma and medial basal hypothalamus of the rat :*

The relationship between the tanycyte ependyma of the floor and ventrolateral walls ( $\beta 2$ ,  $\beta 1$ ,  $\alpha 2$ ,  $\alpha 1$ .) of the third ventricle and the hypophysial adrenocorticotrophic function and gonadal hormones was studied by Akmayev *et al.*(1978) in the rat. Dexamethasone treatment for 8 days showed increased activity in the  $\beta$ -tanycytes. Golgi apparatus was active and there was an increase of polyribosomes and lipid inclusions. Type I and type II vesicles increased in number. Type I vesicles were 40-50 nm in diameter with smooth contour and moderately dense core. Type II (40-90 nm) coated vesicles had electron-lucent centre. After bilateral adrenalectomy type I vesicles in the Golgi area decreased in number in  $\beta$ -tanycytes. No change in the number of lipid inclusions could be observed. Type III vesicles were frequent. They resembled catecholamine vesicles in appearance and size. Most marked increase in type IV vesicles occurred. They had irregular rosette-like structures 50-140 nm in size, with a small quantity



of dense material between the light center and the limiting membrane. "The authors believe that the correlation between  $\beta$ -tanycytes and hypophysial-corticotrophic activities is regulated by a feedback mechanism and provide evidence for the existence of a coupling servo-mechanism between the  $\beta$ -tanycytes and the hypophysial-adrenocortical system. It is therefore logical to suppose that the  $\beta$ -tanycytes contribute to the inhibiting control of ACTH secretion..... either  $\beta$ -tanycytes secrete some factor that suppresses ACTH secretion, or they exert an inhibiting effect through intensification of their transport activity."

Differences between females and males in the activity of some dehydrogenases (GDH, LDH, G-6-PD, GPDH, NADH<sub>2</sub>-DH) were observed in  $\beta^1$ -tanycytes on the 3rd, 5th and 7th post-natal days. These tanycytes are involved in some way (occurrence of corresponding receptors for the gonadal hormones) for sexual differentiation of the hypothalamus. There was an increase of aldehydefuchsin-positive lysosome-like structures in  $\alpha 1$  and  $\alpha 2$ -tanycytes of male and female rats at 1-3 weeks of post-natal life.

### *The endocrine hypothalamus*

Knigge *et al.* (1978) defined the endocrine hypothalamus to be "that control subsystem containing, among other neural elements, those projections of the peptidergic neurohormone system whose activity provides for the regulation of adenohypophysial function". The peptidergic neurohormone system comprises : (1) Peptidergic hypothalamo-neural lobe projections (vasopressin supraoptico-neural lobe; oxytocin paraventricular-neural lobe; SRIF hypothalamo-neural lobe); (2) Peptidergic hypothalamo-median eminence projections (LRF field I-median eminence; LRF field II-median eminence; SRIF hypothalamo-median eminence; TRF hypothalamo-median eminence; vasopressin supraoptico-median eminence); (3) peptidergic hypothalamo-OVLT projections (LRF field II-OVLT; SRIF hypothalamo-OVLT); (4) peptidergic hypothalamo-hypothalamic projections (SRIF hypothalamo-ventromedial; TRF hypothalamo-ventricular; LRF hypothalamo-ventricular; SRIF hypothalamo-ventricular); and (5) peptidergic hypothalamo-mesencephalic (LRF hypothalamo-tectal; LRF hypothalamo-tegmental).

Hokfelt *et al.* (1978) described aminergic and peptidergic pathways in the nervous system with special reference to the hypothalamus.

Kozlowski *et al.* (1978) studied the neurosecretory supply to extrahypothalamic structures (choroid plexus, circumventricular organs, and limbic system) in the rat. The authors said, "Although highly speculative, it is possible that the bipolar nature of the cell body and its processes shown by immunocytochemistry represents a morphologic correlate to the concept that each neurosecretory cell could function as an integrative unit synchronizing activities of various effector sites according to their site of projection and a particular physiologic state of





the animal. Thus, an individual neurosecretory neuron may send fibrous projections to septal areas, suprachiasmatic nuclei, and zona externa of the median eminence for mediation of stress responses that result in adrenocorticoid release and the appropriate behavioral response. Whether the same cell provides an intraventricular nerve ending for a wide distribution of hormone via CSF, should remain as difficult to prove as it is to solve the problem of why particular brain structures demand *private* peptidergic input versus humorally distributed routes of neuropeptides."



## CHAPTER 13

### THE PITUITARY OF REPTILES

Anatomy of the reptilian pituitary gland has been described by Wings-trand(1951, 1966) (fig. 13.1), Saint-Girons(1963, 1967, 1970) and Holmes and Ball(1974). The following description is based on their works. The pituitaries of *Sphenodon*, *Chelonians* and *Crocodiles* are in most primitive form. Well developed pars intermedia and pars tuberalis are present in these groups. The pars distalis is more or less distinctly subdivided into a cephalic and a caudal lobe as in birds. Pars tuberalis is very much reduced or absent in *Lacertilians*. In the snakes it is not developed even in the embryos. In *Lacertilians* and snakes, tuberalis tissue does not accompany the portal vessels from the eminentia to the pars distalis. Instead there is a connective tissue string, called the pars terminalis. Many degrees of complications exist in the neural lobe. Most compact neural lobes are found in snakes and the snake pituitary is strongly asymmetrical.

#### *The pituitary of Sphenodon (Hatteria punctata)*

The *Sphenodon* pituitary may be taken as the prototype of reptilian pituitaries. Developmentally an anlage consisting of two lobes is seen, one oral and the other aboral. They are separated by a constriction. The lateral lobes take their origin from the oral lobes.

Intermedia is formed during further development. Wingstrand(1951) compared the structural plan of the adenohypophysis in birds and reptiles. It shows that the caudal lobe of the adult avian pituitary corresponds to the intermedia and the adjacent part of the pars distalis in reptiles because the material in both cases is supplied by the aboral lobe. The cephalic lobe in the avian pituitary corresponds to the rostral part of the reptilian pars distalis, and this part is well developed in reptiles. The lobi lateralis give rise to a pars tuberalis in birds, crocodiles, *chelonians* and *Rhynchocephalia*, but do not form a tuberalis in the snakes, in which they are indistinct already in small embryos, and they are partly or completely reduced also in lizards (Wingstrand,1951).

The pars distalis of *Sphenodon* is elongate, ovoid and anteriorly it is separated from the neurohypophysis by a broad cleft. At the posterior end it bends dorsally and fuses with the intermedia. The pars distalis is subdivided histologically into cephalic and caudal lobes. The pars intermedia has two to several layers of cells on the surface of the neural lobe and these cells penetrate between the lobules of neural lobe. In adult specimens no hypophysial cleft is seen. The pars tuberalis originates in a paired way on each side of the pars distalis. The starting point corresponds to the anterior margin of the interme-



dia. As in birds, the cell strings from each side fuse to form a porto-tuberal

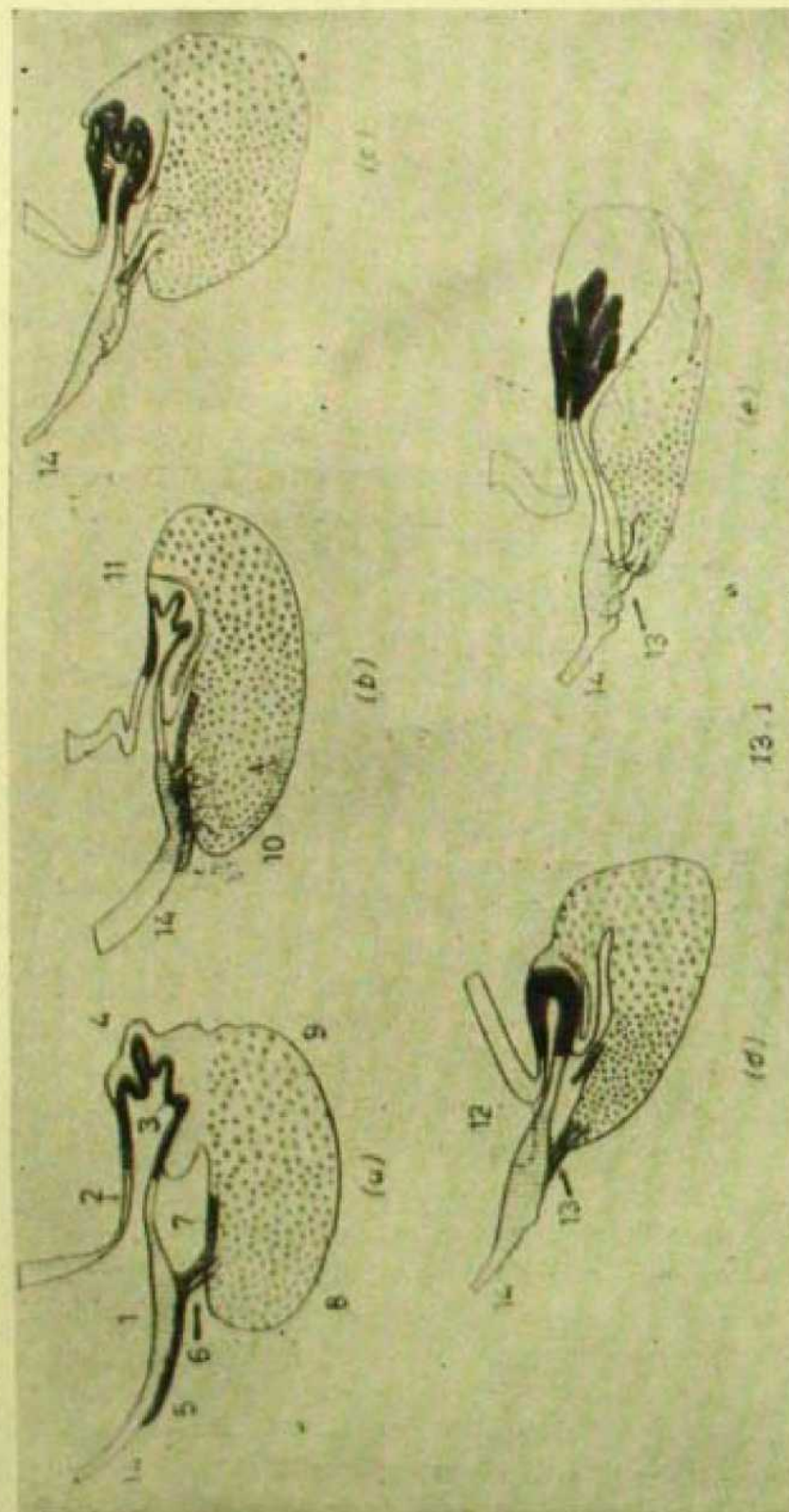


Fig. 13.1. Median sections of reptilian pituitaries: (a) *Sphenodon*; (b) Chelonian (*Testudo*); (c) Crocodile (*Alligator*); (d) Lacertilian (*Lacerta*); (e) Snake (*Python*). 1, Median eminence; 2, Infundibular stem; 3, Neural lobe; 4, Inter-media; 5, Juxta-neural pars tuberalis; 6, Porto-tuberal tract with portal vessels; 7, Pars tuberalis interna; 8, Cephalic lobe of pars distalis; 9, Caudal lobe of pars distalis; 10, (in b), *Zona tuberalis*; 11, Hypophysial cavity; 12, (in d), the situation of the tuberalis plates in *Lacerta*; 13, Pars terminalis; 14, Pars oralis tuberis—From Wingstrand (1966). Courtesy of Professor K. G. Wingstrand and Butterworths, London.





tract together with the portal vessels. The juxta-neural portion of the pars tuberalis covers the median eminence with a few layers of cells and it does not invade the nervous tissue as is noted in *Lacertilians*.

The neural lobe consists of three to four hollow, thin-walled lobules on each side. There are indications to prove that the anlage was bifurcate. The walls of the lobules have an ependymal layer, a fibre layer, and a palisade layer. Free pituicytes are not observed. The median eminence is thin.

#### *The pituitary of Chelonians*

Developmentally the lobi lateralis, the oral and aboral lobes, the constriction between them and the rostral diverticulum appear regularly just as in *Lacerta*. Intermedia is formed by the distal portion of Rathke's pouch proper which is cut off by a second constriction as in snakes. The lobi lateralis are preserved in the adult as a distinct pars tuberalis. The top of the aboral lobe gives rise to an intermedia and the rest of the aboral lobe takes part in the formation of the pars distalis. The remaining part of the pars distalis is formed by the oral lobe (Wingstrand, 1951).

The adenohypophysis of *Chelonians* has three parts: pars distalis, pars intermedia and pars tuberalis. The adenohypophysis is closely attached to the median eminence and therefore the porto-tuberal tract is short, thick and not so prominent as in *Sphenodon* and birds. The pars distalis is histologically subdivided into a cephalic and a caudal lobe in *Testudo mauritanica*. There are plenty of colloid acini in the pars distalis of many *chelonians*. After staining with aldehyde fuchsin, the pars distalis looks almost like a thyroid. The pars intermedia covers the neural lobe from the posterior and ventral aspects and penetrates between the lobules in the medial parts of the intermedia. A few layers of cells are present. Laterally the intermedia is well developed with massive bodies of epithelial strings and acini. Intermedia cells can be faintly stained with haematoxylin and aldehyde fuchsin. A hypophysial cleft is usually present in *T. mauritanica*. The pars tuberalis is usually well developed. The neurohypophysis of most species has a short and illdefined stem, except in *Chelone* where it is long and narrow. The walls of the neural lobe have an ependymal layer, a fibre layer and an outer palisade layer with plenty of neurosecretory substance. Free glia cells are few or absent. A pair of slightly wrinkled sacs may be protruded out from the wall as in *Testudo graeca* or may form many small, hollow lobules as in *Emys* or *Chelone*. The median eminence contains neurosecretory substance in the deep fibre layer and also in the superficial palisade layer.

#### *The pituitary of Crocodilians*

The adenohypophysis appears to be very similar to that of the chelonians. The pars distalis is histologically subdivided into a cephalic and a caudal lobe as



in birds. The pars intermedia is well developed and surrounds the neural lobe and penetrates between its lobules. In young specimens only there is a hypophyseal cleft. The pars tuberalis is thin and paired with a portal zone. The neurohypophysis has a narrow stem. The neural lobe is much lobulated with hollow lobules but dorsally a large sac is present in *Crocodylus*. There are many pituicytes. The capillaries are not situated deeply but they are restricted to the furrows on the surface. The median eminence is very thick and covers only part of the post-optic hypothalamic floor. Plenty of pituicytes and deep folds for the lodgement of the capillaries are noted.

### *The pituitary of Lacertilians (fig. 13.2)*

There is considerable variation in the anatomy of the pituitary gland. Developmentally the pituitary anlage is U-shaped having the epithelial stalk attached to the bottom of the U. The caudal leg of the U contacts the developing neural lobe and so it is the aboral lobe with the aboral lumen. A constriction of the lumen separates it from the oral lumen and this has given rise to a large anterior diverticulum (*Vorraum*). Near the constriction, the lobi lateralis start from the oral lobe as is found in birds. The only difference with the birds is the *mighty* development of the anterior process (as is shown in the left leg of the U in the figures). In avian embryo this diverticulum is small or may be indicated by a solid zone with intensive proliferation.

The pars intermedia is formed by the flattened top of Rathke's pouch. Pars distalis is formed by the rest of the anlage except the lobi lateralis. The lobi lateralis grow out and reach the brain but later on they are reduced to two cell masses which are embedded in the surface of the brain and have lost contact with the main gland (Wingstrand, 1951). The portion of the lateral lobe passing through the portotuberal tract disappears and is reduced to a pars terminalis containing vessels and connective tissues. Szentagothai and Szekely (1958), Szentagothai *et al.* (1962) and Enemar (1960) also observed a very remarkable transformation of the juxta-neural tuberalis plates in most species. Wingstrand (1966) found the cell plates to be situated in the sulcus tubero-infundibularis on each side in *Lacerta*, *Anguis*, *Tarentola*, *Gambelia*, *Uta*, *Sceloporus*, *Uma*, *Streptosaurus* and *Xanthusia* with the disappearance of the basement membranes and the pia under them. The tuberalis cells have entered into the adjacent hypothalamic wall. These cells are difficult to distinguish from neurons. There were no tuberalis cells in *Varanus*, *Chameleon*, *Zonorus* and *Chemidophorus* (Wingstrand, 1966).

In *Xanthusia* the gland is asymmetrical and the pars distalis is shifted to the left and the neural lobe with the intermedia is shifted to the right. This asymmetry is noted in snakes. Pituitary of *Xanthusia* and *Anguis* is flattened dorsoventrally like the pituitary of snakes and in other lizards it is thick. Histological subdivision of the pars distalis into cephalic and caudal lobe is there.



The pars intermedia covers the neural lobe on the posterior, lateral and ventral aspects and partially and incompletely on the dorsal aspect. The pars intermedia may have only one or a few layers of cells as in *Lacerta*, *Anguis* and

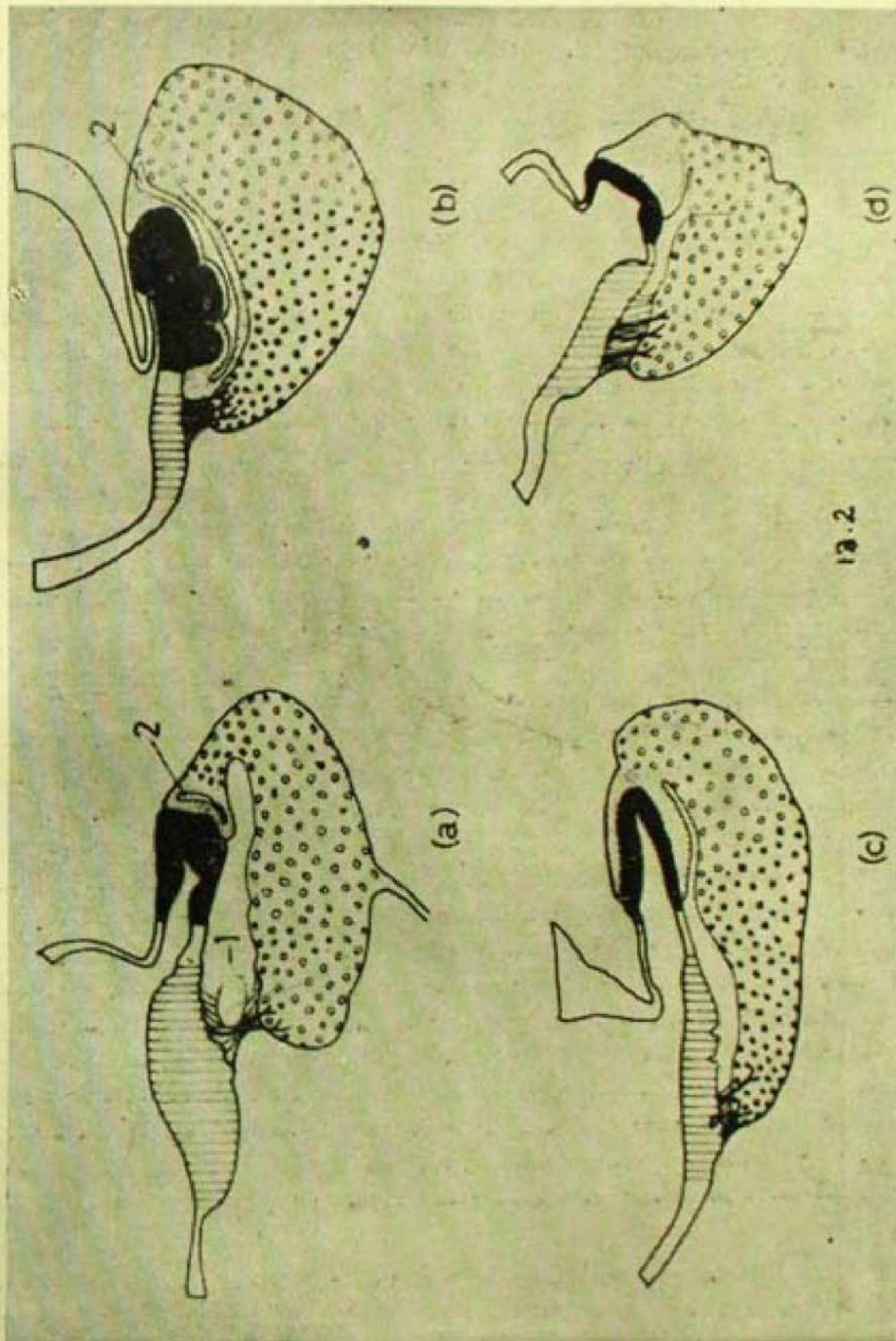


Fig. 13.2. Different types of Lacertilian pituitaries: (a) *Uta stansburiana*; (b) *Varanus niloticus*; (c) *Agama* sp.; (d) *Chameleo gracilis*.—From Wingstrand (1966). Courtesy of Professor K. G. Wingstrand and Butterworths, London.



*Xanthusia* but very thick and large in *Iguanids*, *Chameleon* and *Geckonids* (fig. 13.3). In *Iguanids* the thick intermedia has many layers of cells. The hypophysial cleft is distinct in some groups, small and reduced in others and

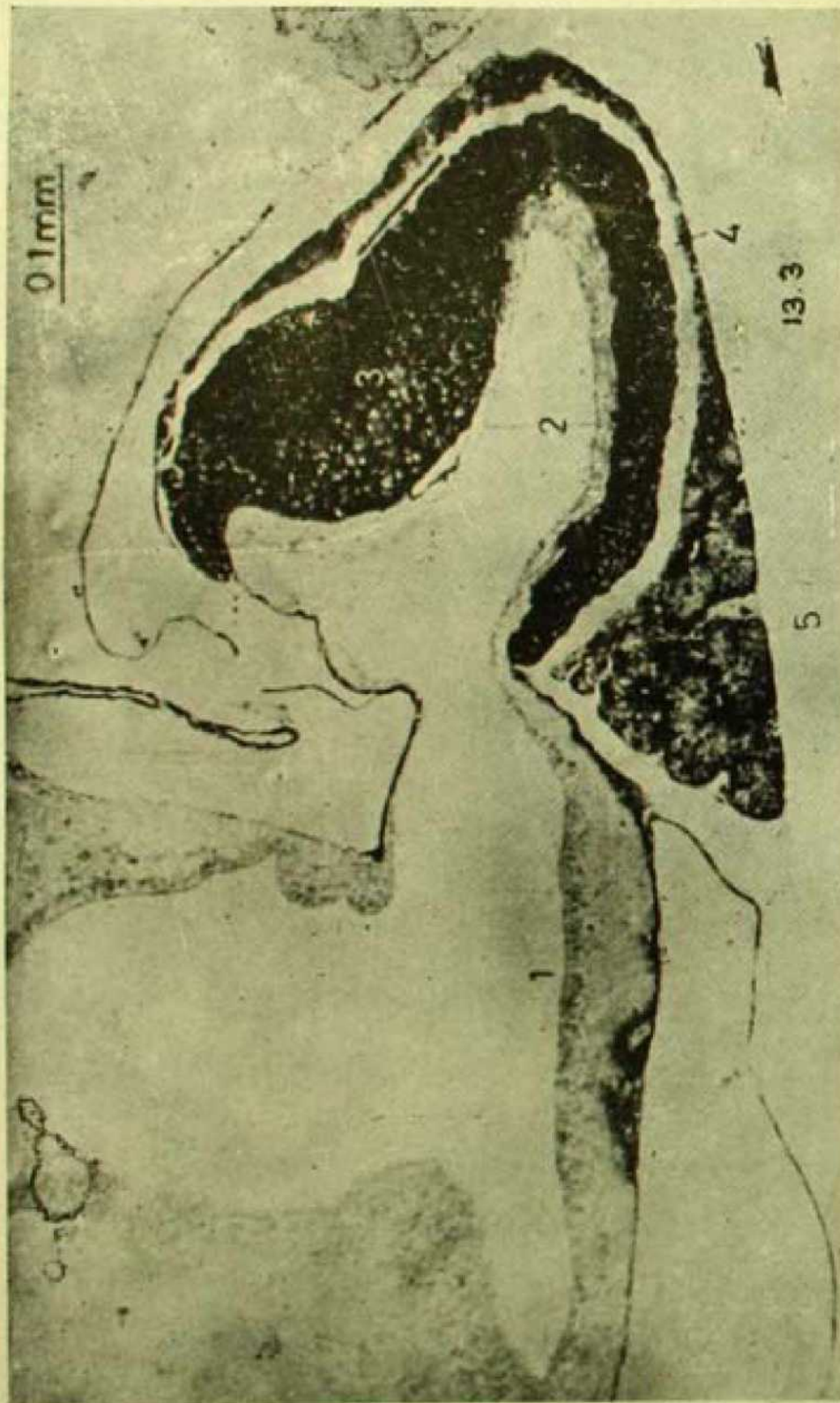


Fig. 13.3. Median section of the pituitary of *Tarentola delandii*, showing the slight differentiation of the median eminence (1), the thin-walled neural lobe (2), the large intermedia (3) and hypophysial cleft (4). 5, Pars distalis.—From Wingstrand (1966). Courtesy of Professor K. G. Wingstrand and Butterworths, London.



completely absent in *Iguanids*, *Varanus*, *Agama* and *Zonorus*. Intermedia cells may lie in the dorsal and ventral walls of the cleft as are found in *Chelonians*.

The neurohypophysis has a long or short infundibular stem or the stem is absent in *Chameleon*. The neural lobe may be thin walled and folded as in *Sphenodon* or lobulated with reduced lumen and thick walled. The *lacertids* have few lobules. Often only the original pair of primary branches of the saccus infundibuli give rise to a large neural lobe of the adult by increase in thickness of their walls. The neural lobe is never compact and the infundibular recess ramifies into the primary branches and other lobules. The median eminence of small lizards is thin with few pituicytes and a smooth surface but in larger species it is thick having numerous pituicytes and the portal vessels are buried in deep furrows on the ventral aspect. The median eminence is situated in a small ventral area at the anterior end of the pars distalis and it is separated from the optic chiasma by a large pars oralis tubercis.

#### *The pituitary of Ophidians (Snakes)*

The pituitary glands in *Typhlops*, *Bitis* and to some extent, *Python* are symmetrical. In other snakes the pituitary gland is asymmetrical. The pars distalis is situated on one side and the neurointermediate lobe on the other (fig. 13.4). In any one species both right-handed and left-handed glands are met with (Wingstrand, 1966). Asymmetry develops late in embryonic development, just before hatching and the pituitary of young embryos are strictly symmetrical.

Asymmetrical snake pituitaries are flattened dorsoventrally. The posterior end of the pars distalis arches over to the opposite side and fuses with the pars intermedia. No hypophysial cleft is met with except in the symmetrical pituitary of *Bitis*.

No pars tuberalis is found in adult snakes. A vascular contact is established by the processus anterior with the median eminence. This process develops into the rostral part of the pars distalis. It is drawn towards one side in old embryos. The pars terminalis is formed by stretched-out portal vessels and connective tissue. In the adult these vessels pass obliquely from the median eminence to the pars distalis.

The pars distalis is histologically subdivided into a cephalic and a caudal lobe. The cephalic lobe contains strongly carminophilic A1 cells.

Pars intermedia is not found in burrowing *Typhlops* and *Leptotyphlops*. No intermedia cells have been found in them. In most snakes the intermedia is massive and forms a thick cup around the posterior end of the neural lobe and the hypophysial cleft is absent.

The infundibular stem of the neurohypophysis passes obliquely from the median eminence and the neural lobe is displaced to the opposite side. The neural lobe is compact in all snakes. The lobules are often solid with connective tissue septa and plenty of pituicytes. The infundibular recess extends into



the base of the lobe and there are few narrow diverticuli. Commonly two pouches are found and they correspond to the paired primary branches of the saccus infundibuli.

The median eminence is situated in the anterior infundibular floor and it may be slightly shifted towards the side where the pars distalis is situated. The

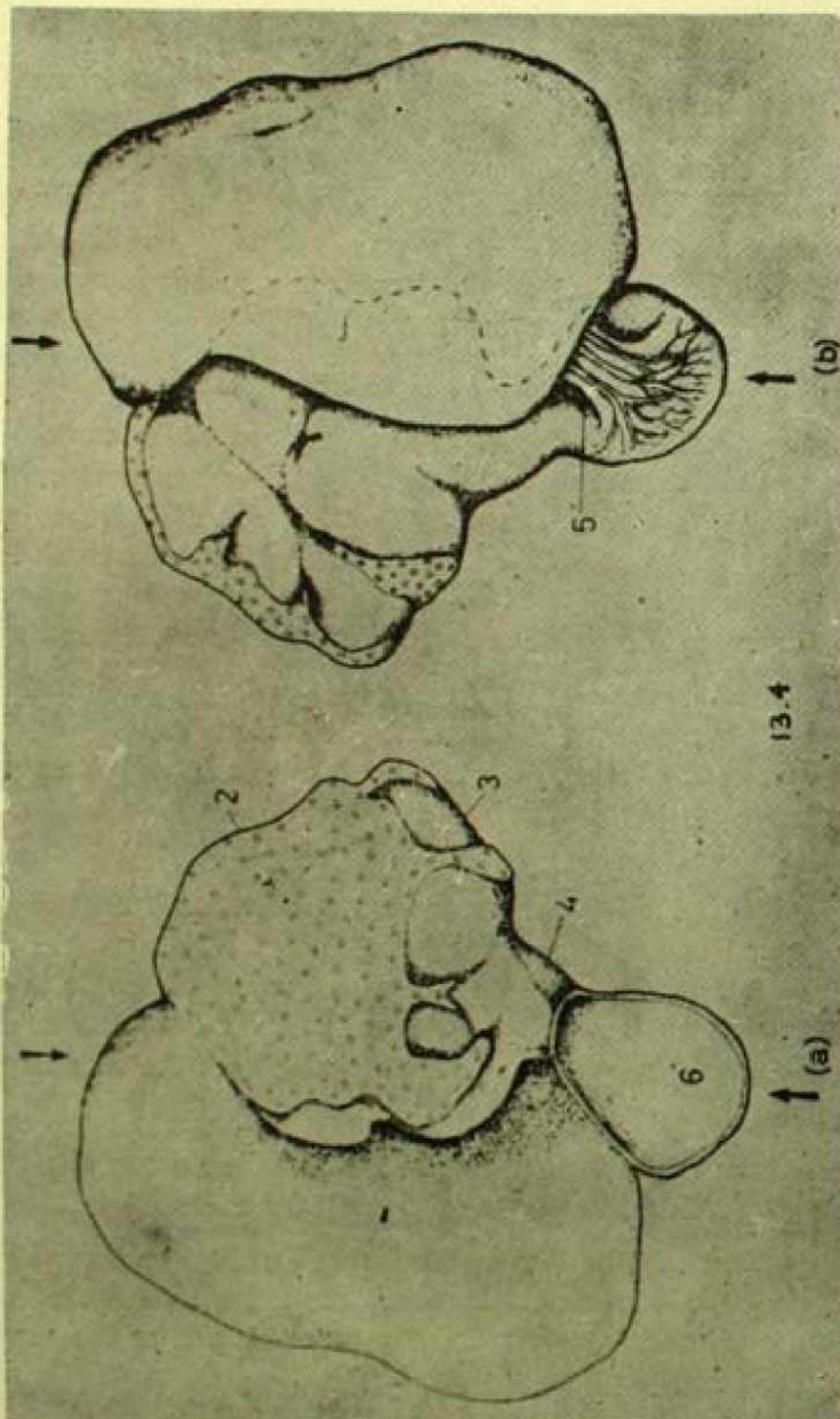


Fig. 13.4. The pituitary of *Vipera berus*, seen from the dorsal (a) and ventral (b) side to show the asymmetry. The arrows indicate the median plane. 1, pars distalis; 2, pars intermedia; 3, neural lobe; 4, infundibular stem; 5, pars terminalis; 6, median eminence.—From Wingstrand (1966). Courtesy of Professor K. G. Wingstrand and Butterworths, London.



median eminence of the snake is always thick, proliferated and free glia cells are present. The portal vessels are in the deep furrows on the surface.

The hypophysial cleft is present in the symmetrical gland of *Bitis* and also in the asymmetrical pituitary of *Vipera aspis*.

### *Vascular supply of the Reptilian pituitary gland*

In all amniotes the portal system consists of a dense plexus of capillaries on the median eminence and they are drained by wide portal vessels which pass to the secondary capillary net of the pars distalis. Wingstrand(1951) found the portal system in the pituitary of snakes (*Vipera*, *Tropidonotus*, *Coronella*) and lizards (*Lacerta*). The primary plexus of the hypophysio-portal system covering the median eminence is formed by arteries coming from the anterior ramus of the carotis far from the point of division in snakes and lizards. In snakes (*Tropidonotus* and *Vipera*) the neural lobe has a double supply. One small artery (fig. 13.5) comes from the infundibular artery and runs along the stem to the neural lobe. Another artery for the neural lobe leaves the carotis just as it enters the sella.

Roy(1958) found that the garden lizard (*Calotes versicolor*) has got well marked median eminence and the primary capillary net of the portal vessels is partly within it and partly on the surface of it. Through the pars terminalis the portal vessels enter into the pars distalis where they break up into secondary capillary net. Basophilic cells akin to the pars tuberalis are located on the surface of the median eminence. Neural lobe and pars intermedia are well developed. The direction of flow of blood in the hypophysioportal vessels is from the median eminence towards the pars distalis. Nerve fibres containing neurosecretory substance are found to end around the primary capillary net in the portion of the infundibulum which corresponds to the median eminence in higher vertebrates. These findings help in the postulation of the idea that neurosecretory substance comes into the pars distalis through the hypophysio-portal vessels and a part contained in the substance stimulates the pars distalis to produce ACTH or gonadotrophin or other hormones. The hypothalamus, median eminence and the hypophysis show marked congestion after the stress of fracture, scald and ether anaesthesia.

(a) In *Calotes versicolor* primary capillaries of the median eminence are fed by the infundibular arteries. These vessels are situated superficially but some loops penetrate deep into the palisade layer. The vascular system of the neural lobe is connected to the vascular bed of the median eminence by a few capillaries. Large portal vessels numbering one or two supply the cephalic lobe of the pars distalis and vessels from this lobe supply the caudal one. The neuro-intermedia receives blood from the neural lobe arteries, branches of infundibular arteries. Branches from them form the plexus intermedius. Pandalai(1966) described the *marginal vessels* in the border zone between the neural lobe



and the pars intermedia of *Calotes versicolor*. These vessels are arranged in the form of simple loops and spirals which protrude into the neural lobe. The neurosecretory endings of the hypothalamo-hypophysial tract bend peripherally and end on the endothelial walls of these capillary loops.

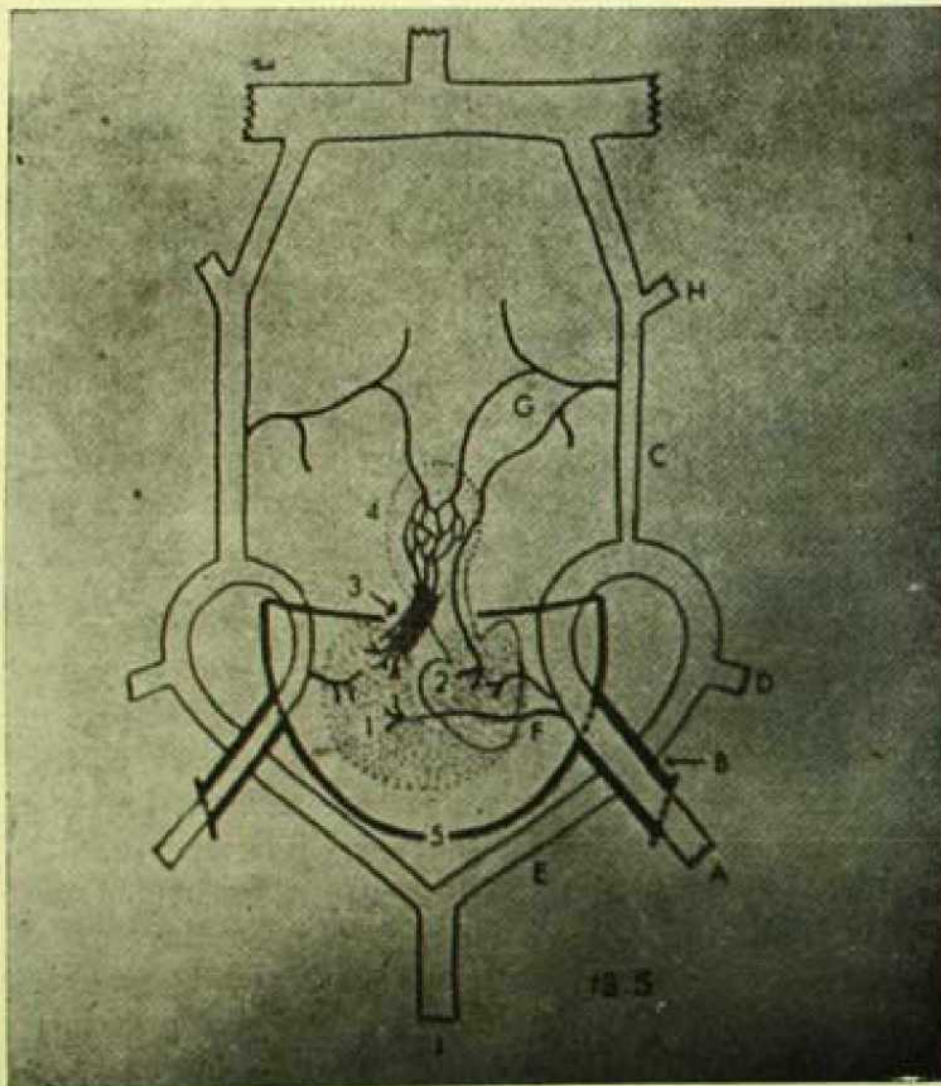


Fig. 13.5. Diagram of the vascular supply of the pituitary in *Vipera berus*. The pituitary is asymmetrical. (A) Carotis interna; (B) the part of the artery situated inside the canalis caroticus; (C) ramus anterior; (D) ramus medius; (E) ramus posterior of the carotis; (F) inferior hypophysial arteries, emitted from the carotis just when it enters the sella; (G) infundibular artery; (H) arteria cerebri media; (J) arteria basilaris. (1) pars distalis; (2) neural lobe; (3) *pars terminalis* with portal vessels; (4) median eminence with the primary plexus of the portal system; (5) internal wall of the sella turcica.—From Wingstrand(1951). Courtesy of Professor K. G. Wingstrand.

These capillary loops give off straight branches from the basal aspects and they supply the pars intermedia. Neurosecretory innervation of the pars intermedia cells has not been observed by Pandalai(1966) in *Calotes versicolor*. NSM circulates in the pars intermedia through blood.



Some of the interlobular and intralobular blood capillaries have connections with the capillaries of the caudal pars distalis. Neural lobe blood passes to the retrohypophysial vein through the capillaries of the intermedia. Veins from caudal pars distalis also join the retrohypophysial vein. There is no separate artery supply to the pars distalis (Nayar and Pandalai, 1963; Sheela and Pandalai, 1966).

(b) The primary plexus of the median eminence is formed by infundibular arteries in *Lacerta agilis* and *Anguis fragilis*. The plexus is situated superficially and there are no vascular loops penetrating deeply. Vascular connections between the primary plexus and the superficial capillary network on the neural lobe surface are there. These connections may be by a *plexus* on the oral surface of the infundibular stem as in *Lacerta* or *several straight capillaries* as in *Anguis*. A Plexus intermedius is formed in the connective tissue septum between the neural lobe and pars intermedia. In these animals neither the neural lobe nor the thin intermedia is penetrated by the vessels. The capillaries are superficial. The superficial vessels are fed by (a) infundibular arteries and (b) capillaries of the median eminence (Enemar, 1960). Blood supply to the pars distalis belongs to the hypophysiportal system. From the primary plexus, four to six portal vessels run into the secondary capillary net in the cephalic lobe of the pars distalis, from where sinusoidal vessels enter into the caudal lobe in the anguid lizards (Saint-Girons, 1967). No direct artery supplies the pars distalis. The venous drainage from the caudal pars distalis and the plexus intermedius is to the retrohypophysial vein (Enemar, 1960).

(c) In the grass snake (*Natrix natrix* L) infundibular arteries feed the primary plexus in the median eminence (Enemar, 1960). Bunches of capillaries penetrate deep into the palisade layer of the eminentia. Seven or eight portal vessels connect the primary plexus with the secondary capillary net in the cephalic part of the pars distalis. Connection between the primary plexus in the median eminence and the vessels of the neural lobe is by only few capillaries. The neural lobe vascular system receives the main feeding vessels from the *hypophysial arteries* and also by a branch from the infundibular artery. Capillary branches from the plexus intermedius pass into the solid lobulated neural lobe and also into the pars intermedia. There is also a superficial plexus. A few small arteries from the internal carotids supply the pars distalis directly. Retrohypophysial veins drain the pituitary. The separation of the vascular beds of the median eminence and neurointermedia which is commonly found in birds and mammals, is not yet complete in the grass snake *Natrix* and this connection is maintained only by a few capillaries.

#### *The reptilian hypothalamus and neurohypophysis*

Roy[1975, from Roy(1976)] discussed the hypothalamus, neurosecretory system, ultrastructure, and experiments on hypothalamus in reptiles with particular



reference to *Calotes versicolor*. The post-optic part of the hypothalamic floor gives rise to the neurohypophysis by an evagination of the posteroventral wall called the saccus infundibuli (Wingstrand, 1966). The base of the saccus infundibuli corresponds to the infundibular stem. The median eminence is located between the chiasma and the saccus infundibuli; but an undifferentiated area remains in between the median eminence and the chiasma called the "pars oralis tuberis" in the majority of reptiles. Majority of the neurosecretory fibres from the supraoptico-hypophysial tract end in the neural lobe having a close contact with the adenohypophysis and this has been called "distale adeno-neuro-hypophysare Kontaktfläche" by Spatz *et al.* (1948) and Spatz (1953). The end of the saccus infundibuli becomes forked or T-shaped and thus produces a pair of primary branches (Wingstrand, 1951, 1959). The growth of the neural lobe takes place by thickening of the walls of the primary branches or nervous material diffusely migrates into the surrounding mesenchyma and thus a thick neural lobe is formed in the snakes and mammals where pituicytes are found. In sphenodon, many reptiles and birds, the neural lobe is thin walled with the presence of ependymal layer and without formation of free glia cells. The wall consists of an ependymal layer, intermediate fibre layer (coarse fibres of preoptic-hypophysial tract) and a superficial palisade layer having endings of neurosecretory axons and crossed by processes of ependymal cells.

#### *Median eminence :*

The median eminence of some lizards is thin with very little proliferation. It has got an inner ependymal layer, intermediate fibre layer and outer palisade layer covered by capillaries. There are few or no pituicytes. Some reptiles have thick median eminence and plenty of pituicytes and capillaries form loops into the wall. Snakes have a typical median eminence without pars tuberalis. Remnants of the pars tuberalis are located in the sulcus tubero-infundibularis on the margin or outside the median eminence in lizards. Wingstrand (1966) therefore said, "The contact with the pars tuberalis is thus a doubtful criterion for defining the eminentia." Vascular contact (portal) between the eminentia and the adenohypophysis is present in the lizard and this forms a *proximale neuro-adenohypophysare Kontaktfläche* of Spatz *et al.* (1948) and Spatz (1953, 1958). According to Spatz there are two zones of contact between the hypothalamus and the adenohypophysis. Pars tuberalis (infundibularis) of the adenohypophysis is closely applied to the ventral surface of the infundibulum. The other contact zone is between the infundibular process and the adenohypophysial pars intermedia. Detailed description has been given by Diepen (1962).

#### *Hypothalamic nuclear masses in reptiles and fibre connections*

(Kappers *et al.*, 1967)

The preoptic area is continuous behind with the hypothalamic area without any break.



The following nuclear masses are noted : the nucleus periventricularis hypothalami, the nucleus hypothalamicus anterior, the nucleus hypothalamicus lateralis and the nucleus hypothalamicus ventralis.

At the anterior end of the hypothalamus deeply stained neurons of the periventricular hypothalamic nucleus fuse with the interstitial cells of the olfactory projection tract. This nucleus is present throughout the whole extent of the hypothalamus.

The lateral hypothalamic nucleus is situated in front of the habenular commissure, ventral to the forebrain bundles and lateral to the periventricular hypothalamic nucleus. Posteriorly it extends slightly behind the habenular commissure. It consists of medium sized cells. Anteriorly this group is continuous as the anterior hypothalamic nucleus. These two groups receive forebrain fibres.

The ventral hypothalamic nucleus is related to the ventral hypothalamic commissural system. At the posterior end of the hypothalamus there is a deeply staining compact nucleus which de Lange called the corpus mammillare because he could trace the fornix bundle to it and it was found to be connected with the anterior thalamic nucleus. It was regarded as the tractus mamillothalamicus or Vicq d'Azyr fasciculus. However, Kappers *et al.*(1967) could not detect such a connection in the Alligator. Diepen(1962) showed in figure No. 82 a and b (page 130) a horizontal section passing through the tuber cinereum and the caudal anlage of the corpus mammillare of *Lacerta viridis*. Bigger nerve cells within the medullated fibres are spoken of as the elements of nucleus mammillaris lateralis of mammals. Possibility of smaller nerve cells lying near the ventricle in a group as anlage of medial mammillary nucleus is there. Similar observations have been made in the mammillary nucleus of *Calotes versicolor*.

A fornix system from the projection cells of the hippocampal region to the hypothalamic areas has been described by many. On the medial wall of the hemisphere the basal olfactory centers are interconnected with the hypothalamic centers by hypothalamic component of the medial forebrain bundle (*C. versicolor*). "The amygdaloid complex, the nucleus of the lateral olfactory tract and the piriform lobe complex are interrelated with preoptic, hypothalamic, and perhaps midbrain areas by way of the stria terminalis and olfactory projection paths" (also in *C. versicolor*). Striohypothalamic component of lateral forebrain fibres proceeds to the lateral and anterior hypothalamic nuclei. Connection of the hypothalamic areas with the tectum by periventricular systems is probable. There are also ascending hypothalamic fibres from lower centres.

#### Neurosecretory system in reptiles

These have been described by Hild(1950, 1951), Diepen(1952, 1955), Bargmann(1954, 1955), Ghiara(1954, 1956 and 1957), E. and B. Scharrer(1954), Roy(1958) and others.



The hypothalamo-hypophysial pathway starts from the supraoptic and paraventricular nuclei in reptilia. The neurosecretory cells extend one of their processes towards the ventricular cavity. The neurosecretory perikarya in Reptilia are not so prominent as noted in Anamniota and the secretory events in them are not so spectacular as noted in Teleostei and in anurous Batrachia (Gabe, 1966). Bargmann *et al.* (1950) noted chrome haematexylin-positive secretion granules in the perinuclear regions of the supraoptic and paraventricular nuclei of Ophidia. Nissl bodies are located at the peripheral regions of the cytoplasm and Dräger (1949) noted vacuoles in the same area, though the significance of them are not yet clear.

The same groups of neurosecretory cells show more or less identical features in Lacertilia.

In chelonians (*Testudo graeca*, and *Emys europaea*) the paraventricular nucleus is very large and long dendrites run from the cells towards the ventricular wall.

The hypothalamo-hypophysial tract is formed by the convergence of the fibres from the neurosecretory cells. They proceed towards the floor of the third ventricle. The ventral wall of the infundibulum corresponds to the median eminence of homoiothermal vertebrates. There are ependymal, fibrous and glandular layers.

Microscopic anatomy of the neurohypophysis in Reptilia shows wide variations (Green, 1951; Saint Girons, 1961). The infundibular recess does not extend into the large neurohypophysis in Ophidia. This lobe is divided by connective tissue septa into small lobules. Mostly in other reptiles the infundibular recess enters into the neurohypophysis to a greater or lesser extent. Digitations proceed from the dorsoventrally flattened sac which contact with the adenohypophysial pars intermedia. The neurohypophysis contains neurosecretory products, terminations of the hypothalamo-hypophysial tract, and pituicytes.

Oksche and Farner (1974) found that in birds numerous nerve cells producing different types of elementary granules were situated outside the classical hypophysiotrophic zone of the tuber and in the parvocellular preoptic, supra-chiasmatic, and anterior hypothalamic regions. Oksche (1978) made a neuroanatomical analysis of the reptilian (chelonian, crocodilian, lacertilian, and ophidian species) hypothalamus. Periventricular rows of nerve cells were observed in the preoptic region of the turtle, *Clemmys leprosa*. At more posterior levels of the hypothalamus there was diffuse lateral accumulation of cells also. There were differences in the distribution pattern of tuberal neurons in turtles (*Geoemyda trijuga thermalis*), crocodiles (*Caiman crocodylus*), lizards (*Lacerta agilis*), and snakes (*Natrix natrix*). In turtles and lizards periventricular rows of neurons dominate. Cluster-like arrangement of neurons is characteristic of medial and lateral tuberal nuclei of snakes (Colubridae, Elapidae). In *Caiman crocodylus*



the periventricular arrangement of neurons is found but local proliferation results in formation of well organized cell clusters in lateral nuclear areas. Oksche(1978) concluded, "In our opinion the cluster-like subunits of hypothalamic nuclei consist not only of cells elaborating different types of neurohormones and neurotransmitter-like substances but also of steroid receptors (hormone concentrating elements), interneurons, intrinsic collaterals and afferents of differing origin. All of these elements are arranged in a patterned manner according to the general morphologic *Bauplan* of the hypothalamus."

Doerr-Schott and Dubois(1978) could localise different peptidergic substances in the brain of reptiles immunohistochemically. LHRH-like substance was found in the median eminence of *Lacerta muralis*. Immunopositive cells were observed in the dorsal cortex of the telencephalon near the anterior ends of the lateral ventricles. LHRH + fibres could not be detected definitely.

SRIF-like substance has been detected in some neurons of the paraventricular nucleus, in the hypothalamo-hypophysial tracts and in the median eminence of *Lacerta muralis*. No immunopositive cell was found in the supraoptic nucleus.

#### *Ultrastructure of the neurosecretory system in reptilia*

Murakami(1961) studied the neurosecretory cells in the hypothalamus of the lacertilian, *Geco japonicus*. The preoptic nucleus of *Geco* consisted of big neurosecretory cells which showed considerable development of ergastoplasm. They contained osmiophilic secretory granules. The clefts between the ns cells and the neuroglial cells or other neurons measured between 100 and 150 Å. The ergastoplasm is irregular; sacs of different sizes occupy the position of the lamellar structure. The sacs contain structures of about 4000Å in diameter. These structures are composed of intense osmiophilic granules of diameters ranging between 900 and 1200 Å. These are thought to be neurosecretory granules. The granules are separated from the ergastoplasmic membranes by a clear space. The Golgi apparatus is juxtannuclearly situated. Cisternae, vacuoles and vesicles have been recognized which contain osmiophilic granular inclusions. The chondriomes are of usual appearance.

Chondriosomes, elementary granules and inclusions as noted in the Golgi complex of the perikarya, have also been noted in the hypothalamo-hypophysial tract. Endoplasmic reticulum and ribosomes have, however, not been noted in this location by Murakami(1961)

Histochemical, electron microscopic and pharmacologic studies on the median eminence of the fish, frog, turtle, bird and laboratory rat were conducted by Kobayashi (1965). AF-positive neurosecretory material has been noted in the fish (*Oryzias latipes*) neurohypophysis and the external layer of the median



eminence of the frog (*Rana catesbeiana*), turtle (*Chelonia japonica*) and birds (pigeon, grass parakeet). Neurohaemal regions exist in the fish neurohypophysis and the median eminence of the frog where axon endings contain large electron-dense neurosecretory granules (about 1400Å) and synaptic vesicle-like structures. A few axon endings are seen here which contain small electron-dense granules (about 800Å) and synaptic vesicle-like structures. The axon endings in the median eminence of the turtle and birds contain few large electron-dense granules but plenty of small electron-dense granules. In the mouse and the rat few aldehyde fuchsin-positive neurosecretory axons are seen in the external layer of the median eminence. But there are many axon bulbs containing small electron-dense granules and synaptic vesicle-like structures. Axon endings having large electron-dense granules are rarely found in this layer of the median eminence. Axon endings containing mostly synaptic vesicle-like structures were also thought to be present in the median eminence and the pars nervosa of the animals.

Small electron-dense granules (about 800Å) in the external layer of the median eminence of the mouse and rat may be carriers of catecholamines (Kobayashi, 1965). Granules in the supraoptico-hypophysial tract and in the pars nervosa are much larger than the small electron-dense granules in the median eminence. Large electron-dense granules are neurosecretory granules which carry neurohypophysial hormones. Small electron-dense granules of the neurohypophysis of the fish and of the external layer of the median eminence of the turtle and birds may be carriers of catecholamines. Some axon terminals in the median eminence may contain both catecholamine granules and synaptic vesicle-like structures containing acetylcholine. Arrangement of the synaptic vesicle-like structures may vary from diffuse type to cluster type in the axon bulbs; clusters aggregate against the inner surface of the membrane of the axon bulb which is situated against the pericapillary connective tissue space. "These active points may be involved in the permeability changes of the membranes of the axon bulbs through the release of ACh, thus facilitating, directly or indirectly, the passage of neurohypophysial hormones or catecholamines through the membrane." Bulbs containing few empty large or small granules have smaller number of synaptic vesicle-like structures whereas bulbs containing many large or small electron-dense granules are associated with many synaptic vesicle-like structures.

Kobayashi (1965) thought that the adeno-hypophysial hormone-releasing factors are present in granules of the same size as either the large or small electron-dense granules.

The neurohypophysis in Reptilia (ophidian *Natrix natrix*) contain unmyelinated fibres with cytoplasm containing elementary granules of diameter ranging from 1500 to 3000Å, chondriosomes and neurofilaments (Bargmann, Knoop and Thiel, 1957). The cytoplasm of the pituicytes has much larger inclusions which have a tendency to disintegrate and thereby produce lamellar formations.



*Experiments on the hypothalamus of reptiles*

Callard and Willard(1969) studied the effects of intrahypothalamic beta-methazone implants on adrenal function in male *Sceloporus cyanogenys*. The findings suggest that the hypothalamus contains steroid-sensitive receptor cells which control adrenal size through the anterior pituitary gland.

Plasma corticosterone levels in the male iguanid lizard *Sceloporus cyanogenys* were noted by Daugherty and Callard(1972) under various physiological conditions. Control baseline levels of the steroid in plasma were significantly decreased by hypophysectomy, adrenalectomy, hypothalamic lesions and cyano-ketone. Levels were increased after treatment with mammalian ACTH in both intact and hypophysectomized lizards.

*Experimental results in Calotes versicolor(personal observations) Roy(1975)*

Experimental conditions	Plasma corticosterone level
1. Mammalian ACTH(2 IU/animal)31°C	Rise at $\frac{1}{2}$ hr.
2. ACTH injection in hypophysectomized animals (8 days).	Rise at 1 hr. from a low level.
3. ACTH in dexamethasone-blocked animals.	Rise at $\frac{1}{2}$ hr.
4. Metyrapone injection (total 60 mg. in 7 days).	Hypertrophy of adrenal glands.
5. Hypophysectomy ... ..	Fall
6. Adrenalectomy ... ..	Fall
7. Betamethazone implants in the hypothalamus (ventromedial nucleus and infundibular nucleus) and median eminence.	Fall
8. Stress (fracture of rt. femur*)	Rise
9. (a) Stimulation of the hippocampus ...	Fall
(b) Stimulation of the hippocampus +stress(*).	Rise(insignificant)
10. (a) Lesion of the hippocampus ...	Rise
(b) Lesion of the hippocampus+stress (*).	Further rise
11. Stimulation of ventromedial nucleus, infundibular nucleus and median eminence.	Rise





Experimental conditions			Plasma corticosterone level
12.	Lesion of the abovementioned areas		Fall
13.	Stimulation of septal area	...	Fall
14.	Lesion of septal area	... ..	Rise
15.	Stimulation of archistriatum	...	Rise
16.	Lesion of archistriatum	... ..	Fall
17.	Pituitary grafts in the mediobasal hypothalamus (ventromedial nucleus, infundibular nucleus) and median eminence.		Grafts well maintained and rise in plasma corticosterone level.
18.	„ +stress (*)	...	Further rise

Roy(1958) stressed the importance of the hypophysiportal circulation in the control of the anterior pituitary by the median eminence and hypothalamus with special reference to the adrenocortical function in the garden lizard, *Calotes versicolor* and changes in them after different forms of stress(fracture, scald and ether anaesthesia).

*Calotes versicolor* has got well-marked median eminence and the primary capillary net of the portal vessels is partly within it and partly on the surface of it. Through the pars terminalis the portal vessels enter into the pars distalis where they break up into secondary capillary net. On the surface of the median eminence there are basophilic cells similar to those noted in pars tuberalis. Investigations of Wingstrand(1951) made him believe that the pars tuberalis really has a function. In lizards and birds some cells in the pars tuberalis are packed with argyrophilic granules. The pars tuberalis is functional whenever present, but that its function cannot be essential for the maintenance of the amniote organism.

There is well-developed neural lobe and pars intermedia in the garden lizards (Roy,1958). The direction of flow of blood in the hypophysio-portal vessels is from the median eminence towards the pars distalis. Nerve fibres containing neurosecretory substance are found to end around the primary capillary net in the portion of the infundibulum which corresponds to the median eminence in higher vertebrates. "These findings help in the postulation of the idea that neurosecretory substance comes into the pars distalis through the hypophysiportal vessels and a part contained in the substance stimulates the pars distalis to produce ACTH or gonadotrophin or other hormones."

Roy(1958) noted the neurosecretory substance (CAHP stain of Gomori) in the following situation in *Calotes versicolor*:



- (a) in the neurosecretory cells of the hypothalamus as granules and along the axons of the cells.
- (b) in the extracellular spaces.
- (c) towards the adjoining ventricle.
- (d) upward extension from the hypothalamic level.
- (e) neural lobe of the pituitary.
- (f) in the richly vascularized median eminence region.

Stress led to depletion of neurosecretory substance. The restorative phase occurred after some time. The vacuolar change in the neurosecretory cells is most commonly met with after stress.

The interrenal cells are small and contain lipid. The gland is very vascular. Stress leads to congestion of the organ and loss of sudanophilic substance from the interrenal cells. Vacuolar change in these cells has also been noted after stress.

*Comparison of the neurohypophysis and its innervation in birds, reptiles, mammals and lower vertebrates (Wingstrand, 1951)*

The neural lobe is formed by proliferation of the top of the saccus infundibuli in mammals, birds and reptiles and it is homologous throughout. Green (1947, 1951) defined the median eminence as a part of the neurohypophysis which is coextensive with the primary capillary net of the hypophysiportal system having a typical histological appearance.

Nowakowski (1951) defined the "infundibulum" of the cat as a part of the diencephalic floor which coextends with the pars tuberalis. The "infundibulum" is delimited by a sulcus infundibularis from the surroundings and has a characteristic histological structure differing from the nearby parts of the brain. Dense capillary net is on the surface of it. This area corresponds well with the median eminence as described by Green and Wingstrand (Wingstrand, 1951).

Nowakowski's infundibulum (median eminence) may be coextensive with the pars tuberalis in the cat and some other mammals, but it is not so in all mammals. The pars tuberalis extends high up by the sides of the tuber cinereum in birds but it is frequently absent from the central and most typical parts of the median eminence. Pars tuberalis is not present in snakes, but they have a distinct and typical eminentia with characteristic histological structure and vascularization. Distinct sulcus infundibularis is absent in many reptiles and birds.

Wingstrand (1951) defines the median eminence as that part of the diencephalic floor which is coextensive with the primary plexus of the hypophysiportal system of blood vessels and characterized by a superficial glandular layer.

Well-defined ventral wall of the third ventricle extends from the chiasma (supra-optic decussations) rostrally to the saccus infundibuli caudally. Median



eminence of the adult cat covers most of this surface except a small part just behind the chiasma. Neurohypophysial structure is not found in this part and it is called pars oralis tuberis by Nowakowski(1951). In birds the whole ventral wall from the chiasma to the saccus is differentiated as a median eminence except in the duck and goose, in which case a small pars oralis tuberis is noted just behind the chiasma. In reptiles (lizards and snakes) a very small part of the postoptic diencephalic floor forms the median eminence and therefore the pars oralis tuberis extends more caudally. In the amphibians the pars oralis tuberis is very large as the median eminence is a small portion near the pituitary.

Wingstrand(1951) concluded, "Nowakowski's infundibulum is the same as my eminentia mediana, his radix infundibuli is my transitional zones, his pars infundibularis is my pars tuberalis, and his Zwischenstück is my infundibular stem."

Nowakowski's external or peripheral zone of the median eminence is the same as the glandular zone of Wingstrand and his central zone is the same as Wingstrand's fibre layer and ependymal layer.

Nowakowski's peripheral zone is innervated by delicate fibres from the nearby parts of the tuber nuclei, the nuclei tuberis infundibularis. These nuclei correspond to the ventral parts or more of the nuclei tuberis in birds.

The peripheral zone of the cat median eminence has little or nothing to do with the neurosecretory neurons of the tr. supraoptico-hypophysius, which mainly terminates in the Hinterlappen (neural lobe). The Radix infundibuli is a transitional zone between the infundibulum and the tuber cinereum. The surface of this area has a neurohypophysial structure and the nucleus tuberis is situated in deeper layers.

In the neurohypophysis of the teleosts there are non-neurosecretory and neurosecretory fibres and glia cells. Most of the neurosecretory fibres pass to the meta-adenohypophysis and a few may pass into the pro- and mesoadenohypophysis. Fibres to mesoadenohypophysis are distinct. Diepen(1962) thinks that the anterior ramifications of the neurohypophysis consisting mainly of non-neurosecretory fibres should be considered as modified median eminence (infundibulum) as these form a type of proximal adenoneurohypophysial contact.

In most cases the pituitary gland is supplied by vessels entering around the stem of the neurohypophysis and ramifying along its branches to all parts of the adenohypophysis. Direct arterial supply into the pro and mesoadenohypophysis has also been noted. Vessels may originate from a ring artery around the stem. On the way to the pituitary, some capillaries or capillary system may be in close contact with eminentia-like structures around the stem base or along the ramifications of the neurohypophysis (Wingstrand,1966).

Regarding the homologies of the teleost neurohypophysis he says that the saccus infundibuli of embryos forming the neural lobe of tetrapods is represented



mainly by the saccus vasculosus of adult teleosts. In the caudal ramifications of the teleost neurohypophysis there is neurosecretory substance, suggesting thereby that this part is the functional equivalent of the neural lobe. Thus, when comparing the basic morphological pattern in the neurohypophysial regions of teleosts and amniotes, the neural lobe of amniotes may be homologized with the saccus vasculosus of fish. On the other hand, when the functional system of the neural lobe with its neurosecretory nerve endings is considered the neural lobe of amniotes may be compared with the neurosecretory part of the neurohypophysis of fish, although the latter is situated far in front of the embryonic saccus infundibuli in some species (Wingstrand,1966).

### *The suprachiasmatic nucleus*

It contains vasopressin and its specific neurophysin in the rodent but this nucleus could not be identified in the suprachiasmatic region in man.

In the *teleost* suprachiasmatic (nucleus opticus hypothalamicus) nucleus has been reported. In the *reptilian* preoptic hypothalamus, a diffuse nucleus pre-opticus lateralis can be identified more ventrally. This is the lateral preoptic and lateral hypothalamic regions of Crosby and Showers(1969). The area pre-optica medialis, nucleus suprachiasmaticus, area hypothalamica anterior and nucleus hypothalamicus arcuatus can be considered as periventricular cell population. In the preoptic region of the *avian* hypothalamus, the nucleus preopticus paraventricularis inferior corresponds to the nucleus suprachiasmaticus of Crosby and Showers(1969). The nucleus preopticus medialis, situated dorsal to the optic chiasma may also be a part of the suprachiasmatic nucleus, at least in the reptilian Caiman (Kuhlenbeck,1977).

Degenerating terminals have been noted in the region of the suprachiasmatic nucleus in lemon shark, fish, snake, duck, bird, duckbill platypus, hamster, rat, and in the cat after eye enucleation or optic nerve section (for references see Joseph and Knigge,1978).

### *The hypothalamic—pineal neural circuit (Klein,1979; Moore,1979).*

Axons are projected from the suprachiasmatic nucleus to the retrochiasmatic hypothalamus. The neural circuit passes through the medial forebrain bundle and the midbrain reticular formation to the upper thoracic intermediolateral cell column. Preganglionic input to the superior cervical ganglia of the sympathetic nervous system is provided by the cells in the latter structures. Projections from the cells in the superior cervical ganglion pass into the pineal gland. Fibres form a dense network or plexus in the gland forming synaptic junctions with the pinealocyte or the transmitter substance is released into the perivascular space.

While there have not yet been any elaborate studies regarding the importance of the suprachiasmatic nucleus in the reptiles, a good review of the structure





and functions of the nucleus in the mammals has been made by R. Y. Moore (1979) (In *Endocrine Rhythms*, edited by D. T. Krieger. Raven Press, New York. pp. 63).

### *Structure*

The suprachiasmatic nucleus, as its name signifies, is located just above the optic chiasma and it lies ventrolateral to the third ventricle. 1.6mm×0.6mm in size, the suprachiasmatic nucleus contains cells which have centrally placed nucleus and scanty, lightly staining cytoplasm. The nucleolus is eccentrically located within the nucleus of the cells. The cell types vary in the different parts of the suprachiasmatic nucleus, viz., the rostral one-fourth and the caudal three-fourths. In the caudal part, again, the cells are of varying nature in the dorso-medial and the ventrolateral aspects. The rostral one-fourth receives no visual projections. In the caudal part the visual projections are predominantly located only in the ventrolateral portion. When the suprachiasmatic nuclei of the two sides are considered together, the ipsilateral nucleus receives only half the number of visual fibres than the contralateral nucleus.

### *Connections*

The afferent and efferent connections of the suprachiasmatic nucleus have been widely studied.

There are two confirmed afferent projections. The first of these are the serotonin-containing fibres. The origin of these fibres is in the nucleus centralis superior of the median raphe. Descending fibres from the anterior hypothalamic area (including the anterior part of the periventricular nucleus) and ascending fibres from the tuberal hypothalamic area are also believed to be contributing to the serotonin-containing terminals. The second of the afferent projections is an indirect visual projection from the ventral lateral geniculate nucleus. Apart from these two confirmed projections, two other afferent projections to the suprachiasmatic nucleus are believed to exist. Firstly, there is the possibility of a norepinephrine-containing terminals; these arise in the brainstem and terminate in the dense capsule of the suprachiasmatic nucleus. Secondly, medial cortical hypothalamic projection (arising from the hippocampal formation) possibly gets connections with the capsule of the suprachiasmatic nucleus on its way to the neighbourhood of the arcuate nucleus. This latter projection is important in view of the role of the hippocampus as an adrenal corticoid receptor.

From the study of the afferent connections it appears that the suprachiasmatic nucleus, apart from being influenced by the visual pathway, is also concerned in the transmission of impulse related to serotonin, norepinephrine and corticotrophin-releasing factor.

As regards the efferent fibres of the nucleus, the axons take a dorsal and a caudal course but their ultimate terminations have not yet been confirmed.





Studies, however, have shown that the fibres extend to the periventricular area through the retrochiasmatic region and to the tuberal hypothalamus as far as the caudal border of the ventromedial nucleus. The axons form a dense bundle in the periventricular region rostrally but on reaching the level of the arcuate nucleus they spread into the ventral tuberal area, some of them terminating in the arcuate nucleus and eminence. This ventral tuberal area has been found to send efferents to the lateral hypothalamic area. This latter area has been shown to project directly on the brainstem and the upper thoracic spinal cord (in the intermediate lateral cell column).

From the study of the efferent fibres, which is not as conclusive as the afferents, it appears that the suprachiasmatic nucleus can affect both hypothalamo-pituitary functions and functions dependent upon the descending projections to the brainstem and spinal cord.

*Functions and relationship to circadian endocrine rhythms.*

It has been finally established that the retinohypothalamic projection terminating in the suprachiasmatic nucleus mediates the effects of light in the entrainment of circadian rhythms. It is also believed by many workers on the basis of different ablation experiments, that the nucleus represents an important component of the central neural circadian oscillating mechanisms. Studies on the effects of careful ablation of the suprachiasmatic nucleus have been carried out by many workers with a view to finding out the role of the nucleus in the circadian endocrine rhythms. All the experiments carried on the rodents have shown that ablation leads to loss of circadian rhythmicity. It has also been found by some workers that oestrous cycling and photoperiodic photosensitivity are abolished by suprachiasmatic nucleus ablation in the hamsters. Experiments on rats have revealed that ablation of the nucleus causes loss of oestrous cycling, loss of ovulation, loss of the circadian rhythm in serum corticosterone as well as loss of the circadian rhythm in pineal serotonin N-acetyltransferase. These observations on the oestrous cycle in both the rat and the hamster are of special importance in that it is now evident that the oestrous cycle is possibly dependent upon a series of circadian events. Also, observations on hamsters have shown that testicular responses to photoperiod are blocked by suprachiasmatic nucleus ablation. These observations, viz, the effects on oestrous cycling and ovulation on one hand and the effects on testicular responses to photoperiod on the other, definitely prove that all endocrine rhythms that are dependent upon circadian mechanisms are abolished by ablation of the suprachiasmatic nucleus.

*Median eminence of the reptiles*

The median eminence has ependymal cells, a thick fibre layer having nsm and pituicytes and an outermost palisade layer. The outermost layer is highly vascular. AF-positive axons are plenty in the median eminence. No Cajal-positive fibres have been noted to end in the external part of the median eminence



of the lizard. Rich plexus of nerve fibres are found in the external zone of the median eminence when stained after the silver chromate method of Golgi (Rodriguez, 1972). He could find in the lizard nervous elements interposed between the lumen of the third ventricle and portal vessels. These bipolar cells are situated in the floor and in the ventral end of the lateral walls of the infundibular recess. The cell bodies may be located in the ependymal or subependymal layer. They may be neurons, ependymal cells or a special nervous element. They are somehow involved in the hypothalamo-adenohypophysial interrelationships.

Kobayashi and Matsui (1969) described the chelonian median eminence in the turtle *Clemmys japonica* (figs. 13.6, 13.7, 13.8, 13.9, 13.10). The fiber layer consists of hypothalamoneurohypophysial tract containing nsm and glia cells. The palisade layer is well developed. The median eminence has got an anterior and a posterior part as in the birds. Kobayashi (1975) found ependymal processes in the lizard (*Lacerta tachydromoides*) apparently interposed between the neurosecretory axon endings and the capillaries of the primary plexus. If in the white crowned sparrow (Bern *et al.*, 1966), crested newt (Fasolo *et al.*, 1973), and in the lizard all the neurohormone-secreting axon endings terminate on the ependymal processes surrounding the capillaries, the hormones presumably must pass through or between these processes to enter the capillaries. In this case, the ependymal cells could play a role in the release of neurohormones. Both ependymal processes and neurosecretory axons terminate on the capillaries. Their number varies from species to species (Oota *et al.*, 1974). There are four possibilities :

- (a) the neurosecretory axons secrete neurohormones directly into the capillaries and there is no necessity of ependymal cells for the release of neurohormones into the capillaries.
- (b) ependymal cells mediate in the release of neurohormones into the capillaries.
- (c) ependymal processes store neurohormones temporarily after release from the axons.
- (d) ependymal cells may change the nature of the material released from axons before its final passage into the portal capillaries.

Ependymal absorption of hormones, releasing factors, and other substances takes place from the ventricle and their release into the portal vessels occurs. Presumed monoaminergic axons of the median eminence form synapses with the ependymal cells. The absorptive function of the ependymal cells is under inhibitory control of monoaminergic axons (Kobayashi, 1975).

Kobayashi and Matsui (1969) and Urano (1972) found plenty of AF positive nsm in anterior median eminence of the turtle but with very little of it in the posterior median eminence. Neurosecretory axons containing granules about



120 nm in diameter have contacts with other nerve fibres. Three types of neurosecretory fibres end on the portal capillaries near the surface in the palisade layer of the anterior median eminence.

←



Fig. 13.6. Inner layer of median eminence of the turtle, *Geoclemys reevesii*  $\times 17000$ . Courtesy of Professor Kobayashi and Dr. Tsuneki (1977).

Type A fibres (secretory granules of 120 nm and 150 nm in diameter) carry neurohypophysial octapeptides. Type B (aminergic) fibres have 100 nm granules and they proceed from the infundibular nucleus. There are clear *synaptic vesicles* of 50 nm in diameter in all the three axonal endings. Axonal endings of the



fourth type contain *only* clear synaptic vesicles. They may be cholinergic. Urano(1972) found the posterior median eminence of *Clemmys japonica* to have

←

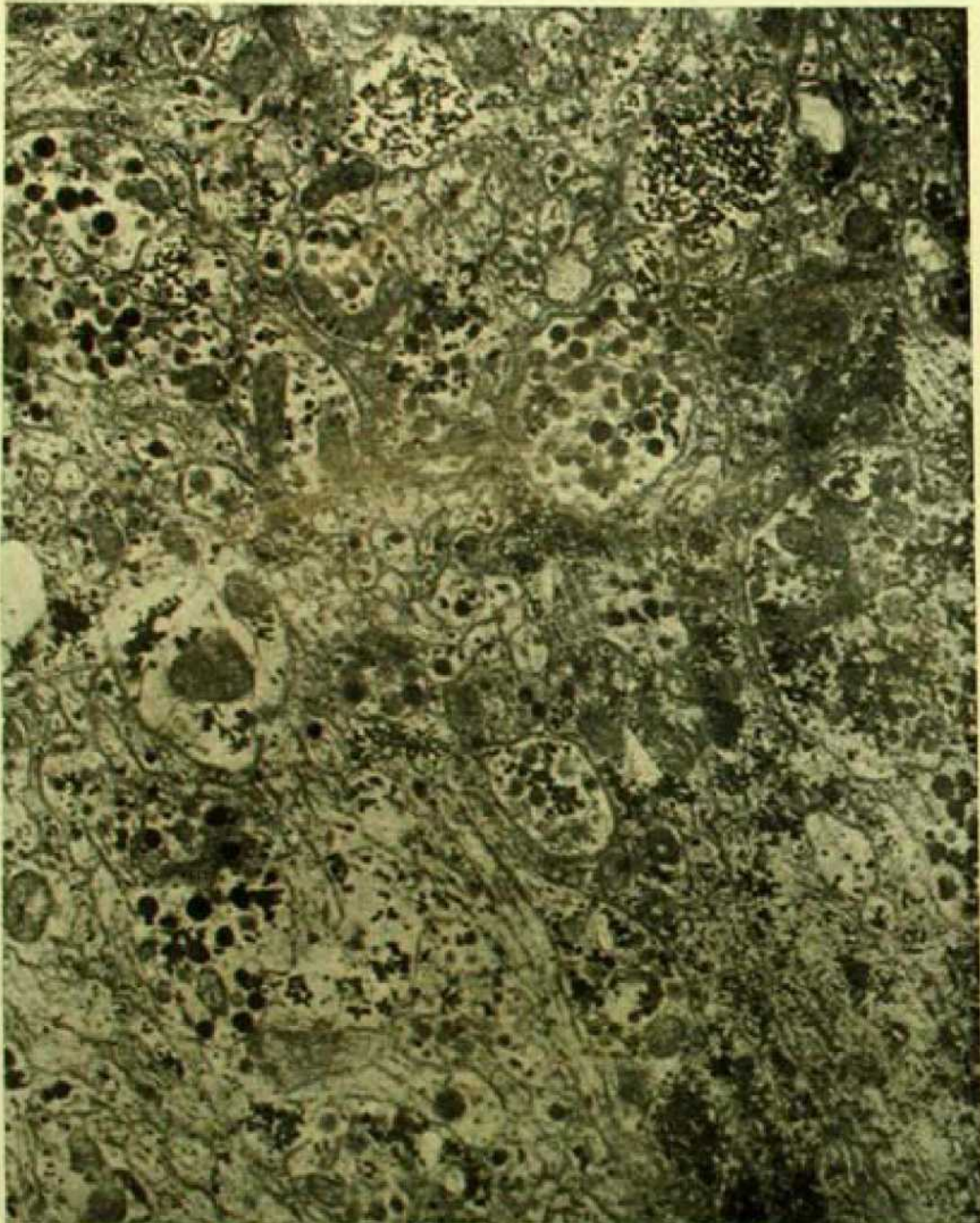


Fig. 13.7. Inner layer of median eminence of the turtle, *Geoclemys reevesii*.  $\times 17000$ .  
 Courtesy of Professor Kobayashi and Dr. Tsuneki(1977).

plenty of monoamine oxidase positive fibres. They end on the primary capillary plexus of this division. Few such fibres are noted in the anterior median eminence.

#### *Neural lobe of the reptiles*

Four types of fibres were described by Rodriguez and La Pointe(1969) in the neural lobe of lizard. There is also a fifth type.



Type I—has small vesicles of 40 nm.

Type II—has dense granules of about 100 nm.

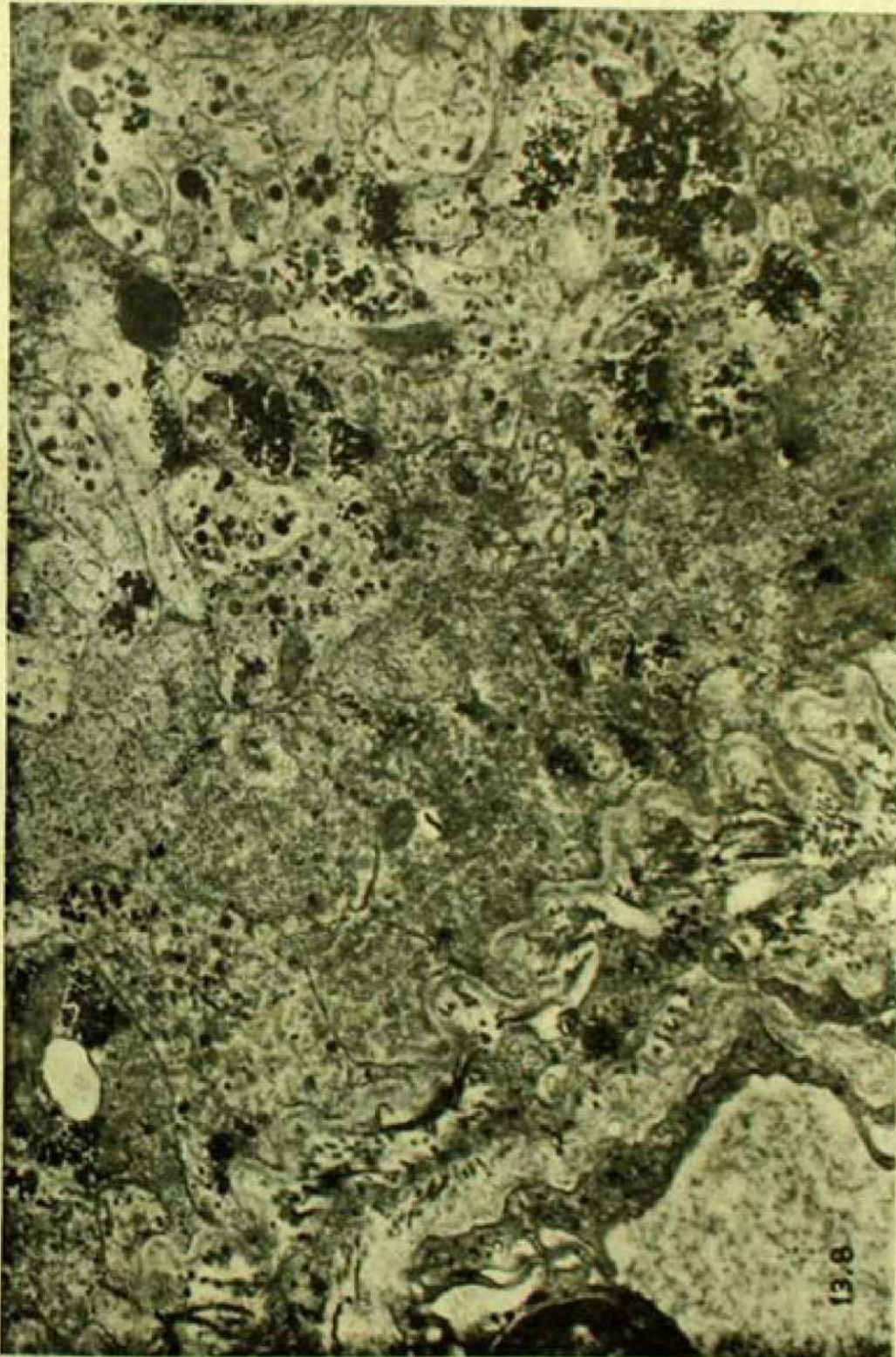


Fig. 13.8. Outer layer of median eminence of the turtle, *Geoclemys reevesii*.  $\times 17100$ .  
 Courtesy of Professor Kobayashi and Dr. Tsuneki(1977).

Type III—contains dense granules of about 150 to 180 nm.

Type IV—has granules of very low electron density and of about 200 nm.



Type V—contains dense granules of about 130 nm (Rodriguez, 1971; in *Klauberina riversiana*).



Fig. 13.9. Outer layer of median eminence of the turtle, *Geoclemys roevesii*.  $\times 17100$ . Courtesy of Professor Kobayashi and Dr. Tsuneki (1977).

Type III axons are most numerous and the ratio between the number of type III and types IV & V varies from 2:1 to 4:1. Type I axons are more numerous than type II axons.



The ependymal processes of the lizard neural lobe are straight and perpendicular to the vascular septum. Perivascular *cuff* is formed by the ependymal process-endings separating the nerve endings from the blood vessels. The cuff is a complete ependymal barrier. Plenty of microvilli are projected into the infundibular recess from the ependymal cells.



Fig. 13.10. Top layer of the median eminence of the turtle, *Geoclemys reevesii*.  $\times 10700$ . Pars tuberalis is seen in the lower right corner. Courtesy of Professor Kobayashi and Dr. Tsuneki(1977).

No connective tissue cells are found in the neural lobes.

The neural lobes of lizard contain both electron-dense and pale neurosecretory granules. They represent two types of granules(Rodriguez,1971). 150



to 180 nm granules store AVT (arginine vasotocin). 130 nm granules are likely to store mesotocin. The third type of granules in the lizard neural lobe (pale granules of 160-220 nm in diameter) store oxytocin.

Weatherhead(1971) described the neural lobe of *Sphenodon*. The neural lobe cavity is lined by ependymal cells. The cells are conical or flattened and they have lipoidal bodies. The ependymal cells have basal processes which in groups cross the neural lobe at right angles or obliquely to enter the neuro-intermedia septum. Ependymal end-feet are formed on the septum. The end feet form a *discontinuous layer* between the neural lobe and the basement membrane of the plexus intermedius. The plexus is formed by branches of the hypophysial artery and there is no capillary supply to the neural lobe. The ependymal end feet form a *continuous layer* in the infundibular stem. Ultra-structurally the ependymal cells have irregular processes and microvilli towards the infundibular recess. The basal processes have mitochondria, fine fibrils and end feet.

A fibre layer is seen under the ependymal layer. Pituicytes are absent and there are plenty of type A1 fibres (secretory granules of 157.5 nm in diameter) and type A2 fibres (secretory granules of 200 nm). The secretory granules in type A2 fibres are less electron-dense and more irregular. Occasionally type B fibres are met with. They have dense core vesicles and there is a space between the granule and the membrane. Diameter of the vesicles is 80 nm. All these fibres contain *synaptic vesicles* (50 nm in diameter) in addition to the secretory granules. Another type of fibre is sometimes met with which contains only electronlucent synaptic vesicles of 50 nm in diameter (type C fibres). In the fibre layer the most preponderant structure is that of non-neurosecretory nerve fibres. They contain neurotubules and neurofilaments. Type A(A1) is more common in the inner palisade layer and types A1, A2, B, C fibres have contact with the pericapillary space of the plexus intermedius. Types A1 and B are the commonest types here and type A2 is rare. Type B fibres are also rare and they have synaptoid contacts with the ependymal basal processes and end feet. Pericapillary space contains connective tissue cells eg. collagen fibres and fibroblasts.

Endothelium of the plexus intermedius is apparently not fenestrated. The endothelial cells contain pinocytotic vesicles, rough endoplasmic reticulum and mitochondria. The neurosecretory material reaches the neural lobe through the capillaries of the plexus intermedius.

Guillette, Jr.(1979) presented the first report of arginine vasotocin (AVT) being used for the purpose of induction of birth in a viviparous lizard (*Sceloporus jarrovi*. AVT may take up an active role in reptilian oviposition or parturition. AVT and mesotocin have been detected by Acher *et al.*(1972) in *I. iguana*. Oxytocin has not been isolated by them.



Vasotocin immunoreactive neurons were observed by Gabrion *et al.* (1978) in the perichiasmatic area of *Natrix viperinus* and *Malpolon monspessulanus*. These neurons were also found to be aggregated in front of the optic chiasma. Reactions were more prominent in *N. viperinus* than in *M. monspessulanus*. Immunofluorescent axons were situated in the superficial ventromedial hypothalamus. In the rostral median eminence the immunoreactive external bundle reached the surface of the median eminence. The most internal bundle extended to the neural lobe through the caudal median eminence. Strong fluorescence was noted in the neural lobe.

### *Reptilian pars intermedia*

MSH is produced in this part of the gland. Wingstrand (1951) observed considerable differences in the cytology of the intermedia when different species of lower vertebrates were compared. Wingstrand (1966) said, "This is hardly in harmony with the common belief that MSH production is the only function in all forms. It may, on the other hand, indicate that our cytochemical and cytological methods emphasize differences which are of little importance for the production of melanophore-dispersing substances."

Intermedia cells are usually polygonal or prismatic but slender ependyma-like cells are present along with rounded cells in some mammals. This is the prominent cell type in gecko (*Tarentula*). There is a vesicular nucleus, nucleolus and mitochondria and they look very similar to the basophils of the pars distalis. Golgi apparatus is present in the lizard *Anolis*. Bargmann, Knoop and Thiel (1957) observed a typical ergastoplasm with densely packed, rough-surfaced sacs in the intermedia cells of the snake *Tropidonotus*.

Zuber-Vogeli *et al.* (1979) found two types of cells in the intermediate lobe of *Uromastix acanthinurus*: one with very few granules and the other with plenty of granules which are PAS+ and mauve coloured with Azan and Cleveland-Wolfe. Mohanty and Naik (1979) observed dark cells and light cells in the pars intermedia of the skink. Both cell types are PAS+ and AB-. Light cells are weakly stained by PbH. Dark cells are moderately PbH+ and MB-. This cell type shows changes after subtotal adrenalectomy and after metopirone administration. It may be related to ACTH secretion.

The cytoplasmic granules are strongly acidophilic in snakes (Sieler, 1936; Wingstrand, 1951; Green, 1951). Similar acidophilic cells have been found in some lizards (*Varanus*, *Agama*) by Wingstrand (1966). The intermedia cells of other lizards are basophilic or chromophobic and Wingstrand (1951) found the intermedia cells of gecko *Tarentula* to be strongly stained by both basic and acid dyes.

Ultrastructurally the acidophilic granules of the snake pituitary are much larger, 4000 to 6000 Å (Bargmann, Knoop and Thiel, 1957). In reptiles these



are concentrated at the end of the cell which has a contact with the basement membrane and there is a marked polarity regarding the distribution of other organelles.

The secretory granules contain glycoproteins. They are PAS+, AF+ and Krezofuchsin and resorcin-fuchsin positive.

Bargmann, Knoop and Thiel(1957) observed large lipoid containing inclusions in the shape of osmiophilic flakes in the intermedia of the snake *Tropidonotus* ultrastructurally.

Pandalai(1966) did not observe neurosecretory nerve fibres or nsm in the pars intermedia of garden lizard (*Calotes versicolor*). NSM circulates in the pars intermedia through blood. Shanta Nayar and Pandalai(1963) found an accumulation of an acidophilic colloid hormone in the pars intermedia when the animal was subjected to controlled light intensities and background colorations. In chronic experiments (6-7 days of continuous illumination against a black background; or 6-7 days after bilateral enucleation of the eyes) the colloid droplets burst through the cell membrane and are freely situated in the extracellular spaces. The acidophil colloid contains MSH.

Degenerative changes have been noted after dehydration in the paraventricular nucleus of the wall-lizard *Hemidactylus brooki*.

Rodriguez, La Pointe and Dellman(1971) did not find AF + nerve fibres in the pars intermedia of the lizard *Klauberina riversiana*. Nerve fibres of any kind could not be observed in the pars intermedia of this species.

Glandular cells of pars intermedia of *Klauberina* showed changes after transection of the infundibulum. Majority of the granules were partially or completely depleted of their electron-dense contents. Amidst these depleted granules there were only a few small and very dense granules. The capillaries of the vascular septum are collapsed in normal animals but they become dilated after the operation. Pars intermedia cells are normally deeply stained with toluidineblue but after transection the staining is very faint. The pars intermedia of animals kept on a black background under constant illumination showed changes identical with those observed after transection of the infundibulum. In the few transected lizards significant dispersion of the skin melanophores occurred only two or three days after the operation.

The reptilian (lizard) intermediate lobes are not innervated. The experiments and findings mentioned above suggest that the lizard pars intermedia is under inhibitory nervous control by fibres coming from the infundibulum. These fibres do not reach the pars intermedia. In the lizard Types A and B fibres would release their active principles into the uninterrupted vascular septum. From this place they would reach the pars intermedia cells. The control thus seems to be neurovascular.





Urano(1972) found monoamine oxidase activity in the pars intermedia cells of the turtle (*Clemmys japonica*) and the fibres in between the cells were aminergic, sometimes having contact with them.

Weatherhead(1978) described the comparative cytology of the neurointermediate lobe of the reptilian pituitary.

Levitin(1980) obtained results to indicate that serotonin (5-HT) is a physiological melanocyte-stimulating hormone-releasing factor in the lizard *Anolis carolinensis*. Levitin(1980) suggested that a serotonergic stimulatory, and a dopaminergic inhibitory, mechanism controls the release of MSH from the pars intermedia of the *Anolis* pituitary gland.

#### *Pars intermedia of rats*

L. A. Sternberger(1974) in his book, "Immunocytochemistry" (Publisher : Prentice-Hall, Inc., Englewood Cliffs, N. J.) (pp. 160-169) stressed the importance of the works of G. C. Moriarty and N. S. Halmi (J. Histochem. Cytochem. 20, 590, 1972, and Z. Zellforsch. 132, 1, 1972). The authors studied the formation and distribution of corticotrophin (ACTH) in the anterior and intermediate lobes of the rat pituitary. They used dilutions of rabbit antiserum to the 17-39 peptide of ACTH ( $\beta$ p17-39) and the unlabeled antibody enzyme method on Araldite sections. Intermediate lobe secretes ACTH and MSH. The  $\beta$ p17-39 ACTH, unlike the whole ACTH molecule, possesses no cross-reactivity with MSH.

ACTH in the anterior lobe and the intermediate lobe can be compared on the same section at the areas where the anterior lobe interdigitates with the intermediate lobe. In the anterior lobe ACTH cells are small in number. All cells of the intermediate lobe contain specifically staining granules except the cuboidal or flattened epithelial cells bordering the lobules. The granules in the stellate ACTH cells of the anterior lobe are distributed at the periphery. These cells are in contact with the capillaries. In the intermediate lobe the secretion granules have no relationship to capillaries because the vascularization of this lobe is poor. The granules are situated at least at one pole of the cytoplasm. The concentration of ACTH per granule is higher in the intermediate than in the anterior lobe. The granules in the anterior lobe are of uniform size. Each granule stains uniformly. The granules in the intermediate lobe are heterogeneous in size and ACTH within individual granules is not uniform. Golgi zone contains ACTH in the anterior lobe, while it is strikingly free of ACTH in the intermediate lobe.

#### *Cell types in the reptilian pars distalis*

Pituitary cytology has been reviewed by Saint-Girons(1963, 1967, 1970, 1978), Grignon(1963), Eyeson(1970), Licht and coworkers(1969-1978), Del Conte(1969, 1972), Holmes and Ball(1974), Fontaine and Olivereau(1975), Doerr-Schott(1976), Mohanty and Naik(1979), and Zuber-Vogeli *et. al.*(1979).



Five different chromophilic cell types are found in the pars distalis. Eyeson(1970) and Holmes and Ball(1974) group them as acidophils type 1, acidophils type 2, basophils type 1, basophils type 2, and basophils type 3. Chromophobes are found in different proportions. They are rare in anigid lizards and plenty in lacertid and iguanid lizards (Saint-Girons,1967; 1970). Mohanty and Naik(1979) however, identified two types of acidophilic (A1 and A2) and four types of mucoid (B1, B2, B3, B4) cells in the pars distalis of the scincid lizard, *Mabuya carinata* (Schneider). B3 and B4 cells were lead-haematoxylin + and only B3 cells were stained by methyl blue. As B2 and B3 cells showed significant hyperactivity after castration, the authors thought that they are involved in gonadotrophin secretion. B3 cells secrete interstitial cell-stimulating hormone (ICSH) and B2 cells secrete follicle-stimulating hormone (FSH). After subtotal adrenalectomy or metopirone administration, B4 cells showed hypertrophy, hyperplasia, and degranulation. These findings indicate that B4 cells secrete ACTH. That B3 cells secrete ICSH has also been observed by Herlant and Grignon(1961), Grignon(1963), Saint-Girons(1963, 1967), Eyeson (1970) and Yip and Lofts(1976). ACTH is secreted by chromophobes (Del Conte,1969, 1972; and Yip and Lofts,1976). Nonnotte-Ferray and Toubeau(1977) found ACTH activity in rostral pars distalis and the cells were well granulated in *Natrix natrix*. They found also ACTH secreting cells in the pars intermedia.

#### *Acidophils type 1 (A1)*

These large carminophilic cells are situated in the cephalic lobe alongside the capillaries. They correspond to X cells of Saint-Girons. The A1 cells form pseudofollicles in *Sphenodon* and some lacertids (Saint-Girons,1963, 1965, 1967). The cells may be finely or coarsely granulated. A1 cells in *Agama* are strongly erythrosinophilic, Luxol fast blue and orange G positive and they are yellow or pale green when stained with combined stains (Eyeson,1970). A1 cells are PAS, AF and AB negative.

Prolactin activity in the rostral zone (Licht and Nicoll,1969) has corroborated the presumptive function of the erythrosinophilic cells (Herlant and Grignon,1961). Prolactin activity is located in the cephalic lobe of *Anolis* (Sage and Bern,1972). In *Agama* weak prolactin activity has been found by Eyeson(1970) in the caudal lobe. These cells do not respond to steroid hormones, thyroxine, thyroidectomy and metopirone and they are unconnected with reproductive cycle. In *Lacerta muralis* Doerr-Schott(1976) found A1 cells of the rostral region to contain LTH by immunohistochemical method. Zuber-Vogeli *et al.*(1979) observed LTH-like cells (A1 cells) in the rostral region of the pituitary of the saharian lizard *Uromastix acanthinurus* by cytoimmunofluorescence technique. A1 cells of the skink *Mabuya carinata* are prolactin secreting cells and are situated in the caudal pars distalis, whereas A2 (STH) cells are rostrally located (Mohanty and Naik,1979).



Prolactin is antigonadotrophic in reptiles (Nicoll,1974). It helps tail regeneration, skin sloughing, and restores plasma sodium levels in hypophysectomized lizard (along with corticosteroids).

Antigonadal effects of prolactin were found by Hensgen *et al.*(1980) in female *Anolis carolinensis*. Prolactin acts on the ovary by suppressing the growth and steroid biosynthesis of smaller ovarian follicles.

### *Acidophils type 2 (A2)*

These cells correspond to alpha cells or acidophils of Saint-Girons(1963) and Grignon(1963) and are plentiful in the caudal pars distalis. Granules of A2 cells may be fine or coarse depending upon the species. After Herlant's alizarin blue tetrachrome, A2 cells are OG+. They are PAS, AF, and AB negative (Eyeson,1970; Saint-Girons,1967,1970). Ultrastructurally the granules in A2 cells of *Lacerta sicula* are 310 nm in diameter(Della Corte, Galgano and Angelini, 1968). Somatotrophic activity (Licht and Rosenberg,1969; Licht and Nicoll, 1969,1971) corresponds in distribution to the caudal somatotrophic cells(Saint-Girons,1961). In viper *Vipera* Grignon(1963) found a correlation to exist between A2 cells and interrenal activity. Eyeson(1970) similarly found such correlation in female *Agama*. No correlation could however, be established in the male. A2 cells secrete STH in *Lacerta muralis* (Doerr-Schott,1976 : immunohistochemical detection by light and electron microscopy) and in *Uromastix acanthinurus* (Zuber-Vogeli *et al.*,1979 : cytoimmunofluorescence technique).

### *Basophils type 1(B1)*

The basophils type 1 cells are delta cells of Saint-Girons(1963). The thyrotrophic cells are situated ventrally in *Cnemidophorus l. lemniscatus* (Del Conte,1972) and in *Testudo* they are caudal in distribution(Herlant and Grignon, 1961). Licht and Rosenberg(1969) confirmed these data by the demonstration of existence of thyrotrophic activity in this area. These cells may be small, large, spherical or elongated. They form a palisade layer surrounding the capillaries of the caudal lobe (Saint-Girons,1967). The secretory granules are PAS, AF, AB (alcian blue) and aniline blue positive. Large bodies in B1 cells of snakes are erythrosinophilic, orange G positive, PAS positive, and Alcian blue negative (Saint-Girons,1967; Eyeson,1970; Forbes,1971). Eyeson(1970) found the secretory granules to be lead haematoxylin positive in *Agama*. From the experiments of Eyeson(1970) it appears that B1 cells are responsible for thyroidal activity in *Agama*. B1 cells secrete TSH in *Lacerta muralis* (Doerr-Schott,1976) and in the Skink(Mohanty and Naik,1979).

Ultrastructurally B1 cells of *Anolis* show seasonal variations. They are inactive in autumn and winter when inactivity is also noted in the thyroid gland. Activity has been noted in spring and summer in B1 cells and the thyroid gland (Forbes,1971). B1 cells are changed into degranulated thyroidectomy cells after



ablation of the thyroid gland. Golgi complex is hypertrophied and the rough endoplasmic reticulum is dilated. The cisternae (RER) contain PAS and AF positive material. The stellate cells in *Anolis* have close association with the thyroidectomy cells. The stellate cells act as a transport system of some substance between the thyroidectomy cells and blood vessels.

After lesion experiments of the median eminence, anteromedial, posterior or lateral areas of the hypothalamus, significant increase in the weight of the thyroid gland in the lizard *Sceloporus cyanogenys* was noted, seven days after the operations (Callard and Chester Jones, 1971). These experiments prove the existence of an inhibitory hypothalamic influence over secretion of TSH.

### *Basophils type 2 (B2)*

B2 cells are usually scattered throughout the gland and they are beta cells of Saint-Girons (1963). Throughout the pars distalis two types of gonadotrophic cells are found in the tortoise (Herlant and Grignon, 1961), *Lacerta* (Della Corte *et al.*, 1968), *Varanus* (Nouhouayi-Besnard, 1966), Squamata (Saint-Girons, 1961) and *Agama* (Eyeson, 1970). They are dispersed in pars distalis similar to the gonadotrophic activity (Licht and Rosenberg, 1969). Licht and Papkoff (1974) found the gonadotrophic activity in *Chelydra* to correspond to two separate and distinct hormones which are LH- and FSH-like. Fontaine and Olivereau (1975) said, "However, one of the processes used to separate mammalian LH and FSH is not effective with chelonians. This suggests that the two chelonian hormones may be closer to one another than are mammalian FSH and LH. Perhaps, then, these two chelonian gonadotrophic hormones could be looked upon as intermediate between the single hormone of certain lower vertebrates and the two mammalian hormones."

The B2 cells are PAS, AF, AB, and aniline blue positive. The large erythrosinophilic, PAS + bodies are similar to lysosomal R granules. B2 cells are gonadotrophs in *Lacerta muralis* (Doerr-Schott, 1976) and in *Uromastix acanthinurus* (Zuber-Vogeli *et al.*, 1979).

Grignon (1963) studied the adenohipophysis of the land turtle (*T. mauritanica*) and of the Viper (*V. aspis*). Gonadotrophic function of beta and gamma cells appears presently to be beyond doubt. In the male, the correlation between the activity of these cells and spermatogenesis, the development of testicular interstitial tissue and mating permitted the conclusion that FSH and ICSH are produced respectively by beta and gamma cells. In the female the interpretation is not so clear. *Castration studies in the two sexes only confirmed the gonadotrophic nature of the beta cells.*

Secretion of FSH and LH in captive green sea turtle *Chelonia mydas* may be regulated independently and Licht *et al.* (1979) thought that "these two gonadotrophins may have distinctive roles in ovarian regulation that differ from



those suggested by hormone therapy studies." Licht *et al.* (1979) found the snake (Colubridae, Elapidae, and Viperidae) gonadotrophins "to be unique among tetrapod gonadotrophins in terms of their biological cross-reactivity, biochemical composition, and immunochemical properties. Data suggest that snakes may only have a single gonadotrophin that does not show a clear homology to either FSH or LH."

$\beta$ -subunit is primarily responsible for the determination of species specificity in response to LH (Licht *et al.*, 1978).

Licht *et al.* (1980) studied serum gonadotrophins (FSH and LH) and sex steroids (estrogen, testosterone, and progesterone) associated with breeding activities in the green sea turtle, *Chelonia mydas* (mating and nesting in natural populations). Gonadotrophins are relatively low during mating and then show fluctuations during nesting cycle. Estrogen was low during both phases of the reproductive cycle. Testosterone and progesterone were more variable. During beaching and nest construction (digging) FSH is not elevated. During laying of eggs FSH increases. LH increases earlier.

Lance and Callard (1978) studied *in vivo* responses of female snakes (*Natrix fasciata*) and female turtles (*Chrysemys picta*) to ovine gonadotrophins (FSH and LH) as measured by plasma progesterone, testosterone and estradiol levels.

Daniels *et al.* (1979) found variability in effects of different species of gonadotrophins: "the relatively high potency of some species of LH may be related in part to increased half-lives."

Saint-Girons (1963) observed that the LH gonadotrophic cells stained almost always with haematoxylin and reacted most frequently with aldehyde fuchsin.

Holmes and Ball (1974) said, "B2 cell is the only gonadotroph in the reptile pituitary, the B3 cell has some other function."

The hypothalamic control over gonadotrophin secretion seems to be stimulatory in nature as evidenced by intrahypothalamic implants of estradiol in *Sceloporus* (Callard and McConnell, 1969) which suppressed ovulation with reduction in weight of oviduct and without affecting follicular development, and by implants of testosterone or estradiol in the median eminence of the lizard (*Dipsosaurus dorsalis*) which inhibited seasonal gonadal maturation (Lisk, 1967). Less effective inhibition was there when the implants were in the basal hypothalamus or pars distalis.

#### Basophils type 3 (B3)

These are  $\gamma$ -cells of Saint-Girons. Holmes and Ball (1974) found the B3 cells of reptiles to be really amphiphils and they stain violet or purple with Azan, trichrome or Herlant's alizarin blue tetrachrome (Saint-Girons, 1963, 1967, 1970; Eyeson, 1970). These cells in general are large, prismatic and disposed



in palisade along the capillaries of the cephalic lobe. The nucleus is spherical and basally situated. Saint-Girons(1963) studied the variations of the adeno-hypophysial cytology in different families. In *Crocodylus niloticus* the cells are stained with haematoxylin. In *Anguillidae* these cells occupy 2/3 of the rostral pars distalis and are feebly AF + and haematoxylin +. In *Geckonidae* the rostral part of the pars distalis is well developed and the caudal lobe is reduced. The LH (B3) cells are small in number, dispersed and ovoid in shape. In *Iguanidae* and *Agamidae* the cells of the distal lobe are organized in long parallel and longitudinal cords (*Anolis*). The number of chromophobes is particularly increased. The LH(B3) cells are few in number and poorly chromophilic. In *Chamaeleonidae* LH(B3) cells are rather rare and poorly chromophilic. In *Trogonophis* B3 cells are haematoxylin positive.

The size of granules in B3 cells is variable. B3 cells are weakly PAS positive and AF positive and AB negative in *Agama*. In many snakes they are AB positive. These granules are iron haematoxylin and lead haematoxylin positive. B3 cells secrete ACTH in *Iacerta muralis*(Doerr-Schott,1976) and in *Uromastix acanthinurus* (Zuber-Vogeli *et al.*,1979 : cytoimmunofluorescence technique).

Grignon(1963) and Saint-Girons(1963) thought that these B3 cells were LH gonadotrophs because there were changes in these cells during annual cycle and changes in gonads. Del Conte(1969) found these cells in *Cnemidophorus* to be strongly activated by metopirone. Licht and Bradshaw(1969) found corticotrophic activity in the same zone containing B3 cells. Holmes and Ball(1974) therefore remarked, "Since only A1 and B3 cells are distributed preferentially in the cephalic lobe and since the former are lactotrophs, this work identified the B3 cells as corticotrophs, and identification was confirmed by the effects of partial hypophysectomy on the levels of circulating corticosterone in *Anolis* (Licht and Bradshaw,1969)."

Roy(1975, from Roy,1976) found that in the pituitary of the garden lizard (*Calotes versicolor*) there are cells at the rostral region which can be stained with OG and lead haematoxylin. Their corticotrophic nature has been established by responses to metyrapone treatment where hypertrophy and hyperplasia have been noted. These cells are distributed along the secondary capillary nets.

Hypothalamic control of B3 cells secreting ACTH has been previously mentioned along with Roy's(1975) observations in *Calotes versicolor*. Roy(1970) found that removal of the hippocampus in *Calotes versicolor* leads to increased blood corticosterone level even in the second week after operation when the appropriate controls show normal values. The area of the brain removed included fascia dentata and dorsal cortex(Ammon's pyramidal cells) of Ariens Kappers(1936). Some of the neural pathways engaged in pituitary ACTH release have also been mentioned. In 1975 it was further shown that stress activates the hypothalamo-pituitary-adrenal axis. Steroid sensitive areas in the ventromedial nucleus, infundibular nucleus and median eminence controlling



ACTH release are noted. Both inhibitory and stimulatory brain areas for such release have been found. That the ventromedial nucleus, the infundibular nucleus and the median eminence are important for ACTH control has also been proved by pituitary grafting experiments. The grafts were active when placed in the infundibular nucleus, median eminence and even in the infundibular recess of the third ventricle adjacent to the median eminence. Plasma corticosterone level was maintained and stress of femur fracture could increase corticosterone level in such graft bearing animals. The ventromedial nucleus, infundibular nucleus and the median eminence are important areas for the control of ACTH secretion and it has been proved by stimulation and lesion experiments in *Calotes versicolor*. Inhibitory areas include hippocampus, and septal area. found that removal of the hippocampus in *Calotes versicolor* leads to increased Stimulatory areas include archistriatum and the hypothalamus. Thus it has been noted that extrahypothalamic areas of the brain in the garden Lizard (*Calotes versicolor*) control ACTH release.

Duggan and Lofts(1979) obtained data to suggest that separate regulatory mechanisms for corticosterone and aldosterone may exist in the sea snake, *Hydrophis cyanocinctus* Daudin, and such a different regulation may be of physiological importance.

The structure of the adrenal gland in Reptilia has been reviewed by Lofts(1978). Callard and Callard(1978) reviewed the physiology. "There is considerable evidence that reptilian adrenal function is controlled by an adeno-hypophysial-adrenocortical axis as in mammals and apparently all other vertebrate groups. Any variations from this basic pattern would seem to be variations in degree rather than kind. Hypophysectomy invariably leads to a decline in adrenal weight, the extent of which varies with the postoperative interval preceding autopsy, and the time of year at which the experiment is performed" (Lofts,1978).

Hypophysectomy leads to a loss of adrenal weight in *Agama agama* and *Natrix natrix* (Wright and Chester Jones,1957) and in male *Dipsosaurus dorsalis* (Chan *et al.*,1970). Adrenal changes could be prevented by injection of mammalian ACTH. Plasma corticosterone level falls after hypophysectomy in *Anolis* (Light and Bradshaw,1969), in *Sceloporous cyanogenys* (Daugherty and Callard, 1972) and in the freshwater turtle, *Chrysemys picta* (Callard, 1975a). In hypophysectomized animals response after ACTH injection is delayed. Hypophysial extracts of the alligator has corticotrophic activity(*in vitro* avian adrenal)(Gist and deRoos,1966). Licht and Bradshaw(1969)found corticotrophic activity to be localized in the rostral part of the pars distalis of *Anolis*, *Pseudemys*, *Caiman* and *Dipsosaurus*.

Adrenal weight was found to be significantly lowered in *Sceloporous cyanogenys* after implantation of betamethasone into the hypothalamus (Callard and Willard,1969). Blank hypothalamic implants and subcutaneous betamethasone



did not show changes in the adrenals, but adrenal glands of lizards with hypothalamic implants of betamethasone were atrophic and reacted weakly to tests for  $\Delta^5$ - $3\beta$ -hydroxysteroid dehydrogenase. Compensatory hypertrophy of the adrenal cortex after metyrapone in hypothalamic betamethasone implanted animals was prevented. In the other groups of animals (blank hypothalamic or subcutaneous implantation) adrenocortical hypertrophy after metyrapone injections occurred. Stereotaxic lesions were made in different areas of the brain of *Sceloporus cyanogenys* by Callard and Chester Jones (1971) in order to find out the mechanisms for the control of ACTH secretion. The response was measured by noting the effectiveness in blocking ACTH secretion as judged by the degree of adrenocortical hypertrophy induced by metyrapone injections. Increase in adrenal weight after metyrapone was not found in animals with lesions of the anteromedial hypothalamus and median eminence. Cortical cells were atrophic. In all other groups adrenal weight was found to be increased after metyrapone. Stimulatory control of the lizard adrenal gland is mediated by the anteromedial hypothalamus and the median eminence (Lofts, 1978). Hippocampus has a checking influence over the pituitary-adrenal-axis of *Calotes versicolor* (Roy, 1970).

Adrenal hypertrophy has been observed in lizards after cyanoketone (inhibitor of  $\Delta^5$ - $3\beta$ -hydroxysteroid dehydrogenase) and metyrapone ( $11\beta$ -hydroxylase inhibitor) (Callard and Willard, 1969; Callard and Chester Jones, 1971). Corticosterone level in peripheral plasma is depressed after dexamethasone (Licht and Bradshaw, 1969). Plasma corticosterone level is depressed after hypothalamic lesions or hypothalamic corticosteroid implantation in *Sceloporus* and *Dipsosaurus dorsalis*. Implants of aldosterone in anterior hypothalamus and median eminence of *Dipsosaurus dorsalis* could also bring down plasma corticosterone level (Daugherty and Callard, 1972; Callard *et al.*, 1975a, b). "Recent experiments in *Chrysemys picta* using a hollow, indwelling hypothalamic probe show that in the presence of corticosterone or aldosterone, plasma corticosterone falls but rebounds after removal of the pellet. A substance that stimulates ACTH production has been detected in hypothalamic—median eminence extracts of *Chrysemys* tested in a homologous pituitary-adrenal system *in vitro*" (Callard and Callard, 1978).

Plasma corticosterone level increased after confinement stress for 5 hours in *Amphibolurus inermis* to  $9.45 \mu\text{g}/100 \text{ ml}$  plasma (summer) from a basal unstressed level of  $2.34 \mu\text{g}/100 \text{ ml}$  plasma (summer) (Bradshaw, 1975). In *Caiman crocodilus* bleeding stress raised plasma corticosterone level (Gist and Kaplan, 1976). Dexamethasone could block the rise after stress.

Repeated removal of blood samples by cardiac puncture in freshwater turtles, *Chrysemys picta* was followed by gradual increase in plasma corticosterone level, but only cardiac puncture had no effect (Callard, 1975a). Response to haemorrhage in turtles was reduced by hypophysectomy but was not completely blocked. Hypophysectomy + dexamethasone had similar effect.



Callard(1975a, b) obtained dose-response increase in corticosterone production from endogenous precursors when a suspension of isolated interrenal cells of *Chrysemys picta* was incubated with synthetic ACTH<sub>1-24</sub>, or porcine ACTH (3rd IWS), or pituitary extracts of *Rana*, *Gallus* and *Chrysemys*.

Callard and Callard(1978) observed that adrenal responsiveness to ACTH is lower in Reptilia than in the rat. It may be due to the effects of temperature. Plasma corticosterone after ACTH remains elevated for a long time in reptiles.

Roy(1958) observed that the adrenal element lies in the dorsal aspect of the adrenal gland in *Calotes versicolor*; but scattered groups or solitary adrenal cells are found in the gland. The adrenal cells are big with a darkly stained nucleus. The interrenal cells are small and contains lipid. The gland is very vascular. Glandular hypertrophy occurs in summer whereas during winter the reverse is true. Stress of femur fracture, scald and ether anaesthesia leads to a congestion of the organ and loss of sudanophilic substance from the interrenal cells. Vacuolar change has been noted in the interrenal cells in haematoxylin-eosin preparations.

Hypothalamic factors for stimulation and inhibition of prolactin secretion have been obtained in the turtle. Neutralized acid extract of the median eminence from male rats, female turtles (*Pseudemys scripta elegans*), and snakes (*Natrix taxispilota*) of undetermined sex was prepared by Fiorindo(1980). A factor is contained in the reptilian median eminence which stimulates the secretion and synthesis of prolactin. "The prolactin—stimulating factor may be indigenous to the hypothalami of a wider range of reptilian species".

TSH secretion is under inhibitory hypothalamic control in *Sceloporous cyanogenys* (Callard and Chester Jones,1971).

Gonadotrophin secretion is under a stimulatory hypothalamic control.

### *Pars tuberalis*

It is absent in snakes, small in lizards and large in *sphenodon*, crocodiles and chelonians. In crocodiles small groups of cells form pseudofollicles along the pituitary stalk. The nucleus is spherical and the cytoplasm is faintly chromophilic. In the tortoise pars tuberalis is in continuity with the rostradorsal part of the pars distalis. Apart from chromophobic cells there are large granulated cells which are PAS, AF, and AB positive. In the serpents (*Boidae*) there are small involuted chromophobic cells in the inferior part of the pituitary stalk (Saint-Girons,1963).



## CHAPTER 14

### THE PITUITARY OF BIRDS

Wingstrand(1951) described the *structure and development of the avian pituitary* in his detailed monograph. The adenohypophysis is formed by Rathke's pouch and the entodermal Seessel's pouch could not be found to contribute to its formation by him in the investigated species (*Larus*, *Riparia*, *Gallus*) (fig. 14.1).

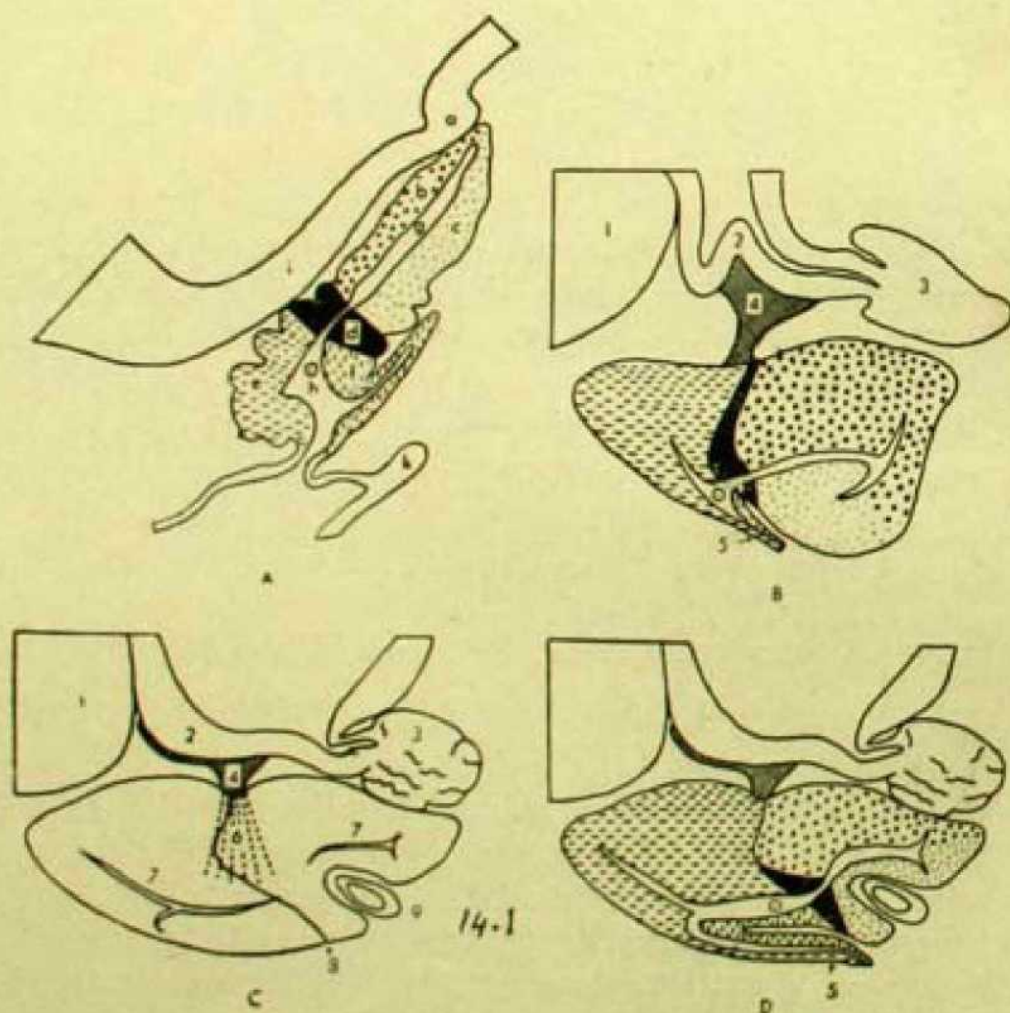


Fig. 14.1. Diagrams showing the development of the adenohypophysis in birds.

- (A) Hypophysial anlage in a 5-day chick embryo (*Gallus gallus*).
- (B) Pituitary of a gull (*Larus ridibundus*, nearly full grown young).
- (C) Pituitary of a partridge (*Perdix perdix*).
- (D) Wingstrand's interpretation of the same gland.

Legends to (A) : (a) saccus infundibuli; (b) rostral wall and (c) caudal wall of the aboral lobe; (d) walls of the constricted part, the narrowed lumen is



In the early stage of development, an aboral and an oral lobe can be seen with a corresponding dilatation of the lumina and constriction between them. An epithelial stalk exists. The lateral lobes have lumina which are continuous with the oral lumen. The contact zone with the brain has been reduced to a small area between the top of the pouch and the growing infundibular process. This area remains single layered upto a fairly late stage of development but a rapid proliferation occurs in other walls with the exception of the epithelial stalk (fig. 14.2). The monolayered wall morphologically corresponds to the *intermedia* of other vertebrates. *Pars intermedia* has not been found to develop in birds.

From this early stage the adult gland is developed in the following way. The caudal lobe in the adult is formed by proliferation from the aboral lobe of the embryo and perhaps also by a few strings from the walls of the constricted part of the anlage. The *intermedia* loses its contact with the neural lobe, proliferates and is included in the caudal lobe. In the adult the cephalic lobe is formed by the oral lobe and possibly by some strings from the constricted part. Massive proliferation takes place from the rostral(dorsal) side of the oral lobe and an anterior diverticulum is situated at this place. This corresponds to the *Vorraum* of *Woerdeman*. Proliferation of the lateral lobes gives rise to the *pars tuberalis*, forming layers of epithelial tissue on the surface of the brain. The epithelial stalk is reduced rapidly and is complete in *Riparia*. However, in *Larus* and *Gallus* the degeneration is characterized by the formation of cysts.

The *pars distalis* is separated from the infundibular stem and the median eminence by a wide cleft which is filled with loose connective tissue. The cleft is bridged by the porto-tuberal tract. The tract passes from the median eminence to the *pars distalis* about halfway between the anterior and the posterior ends of the gland or a little nearer the anterior end. Histologically the *pars distalis* is divided into a cephalic and a caudal lobe. They correspond to the oral and aboral lobes of Rathke's pouch in the embryo.

The *pars tuberalis* originates on each side of the *pars distalis* between the cephalic and caudal lobes. In embryos the lateral lobes grow out near the constriction between the oral and aboral lobes of Rathke's pouch. The inclusion of the base of the lateral lobes in the *pars distalis* can be seen in

stippled; (e) rostral wall and (f) caudal wall of the oral lobe; (g) aboral and (h) oral lumen; (i) the prospective median eminence; (k) Seessel's pouch. The ring with the dot in the centre near (h) marks the point of origin of the lateral lobes as projected in the median plane.

Legends to (B), (C) and (D): (1) optic chiasma; (2) median eminence; (3) neural lobe; (4) *pars tuberalis*; (5) epithelial stalk; (6) *pars tuberalis interna*, projected in the median plane; (7) residual lumen; (8) histological limit of caudal lobe; (9) anastomosis between the carotids.—From Wingstrand(1951). Courtesy of Professor K. G. Wingstrand.



some adult birds eg. *Larus* and *Columba* as a *pars tuberalis interna*. A string of chromophobic and faintly basophilic cells extends from the pars tuberalis proper downwards on each side between the cephalic and caudal lobe (Wingstrand, 1951).

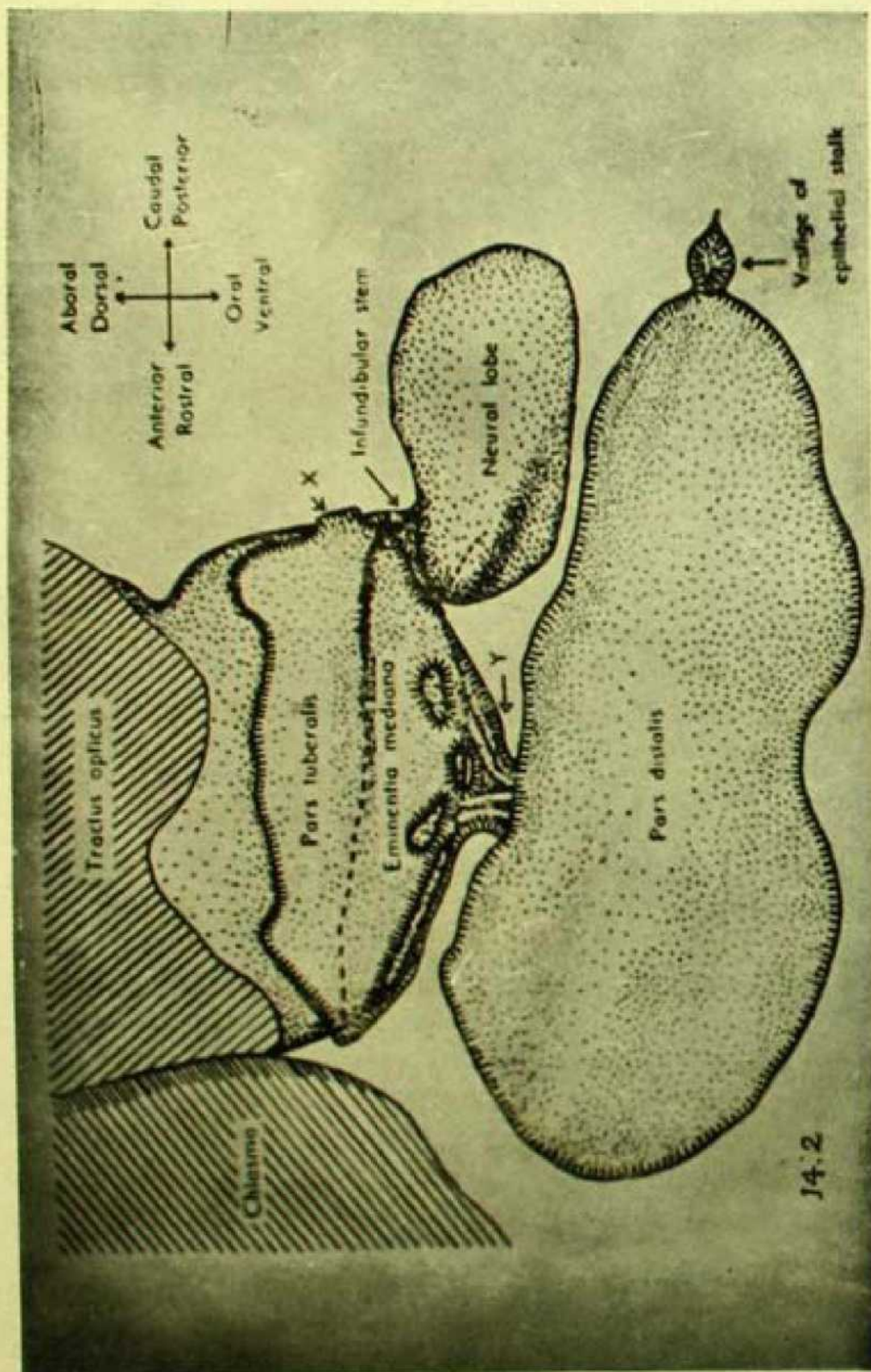


Fig. 14.2. Pituitary of a goose (*Anser anser*). The dorsal limit of the median eminence is indicated by an interrupted line. (X), the junction of pars tuberalis with that of the other side behind the infundibulum. (Y), the connection between the pars distalis and pars tuberalis proper (portal zone).—From Wingstrand (1951). Courtesy of Professor K. G. Wingstrand.



In other birds e.g. *Gallus* this part cannot be easily recognized. The next parts of the lateral lobes fuse to form a portotuberal tract around the portal vessels. The ends of the lateral lobes spread out to form the juxtaneural epithelial layer on the surface of the median eminence and extend upwards along the sides of the tuber. In albatrosses and penguins the tuberalis is very thick having several layers of cells and acini but in majority of birds only a few layers of cells can be found. In *Apus* and *Anser* it is not present in the central parts of the eminentia and the layer on the brain is very thin consisting of only one or a few layers of cells. The descriptions mentioned above are from Wingstrand (1951, 1966).

Roy(1972-1974; from Roy, 1976) reviewed the hypothalamus, hypophysiportal vessels and hypophysis of birds. The author also discussed the brain mechanisms responsible for ACTH release in the pigeon(*Columba livia*) mentioning his own observations.

*Hypothalamus, hypophysiportal vessels and hypophysis of birds*  
*Hypophysiportal vessels in the birds :*

The importance of the hypophysiportal vessels in the control of the anterior pituitary function has been stated in previous chapters. Green(1951) and Hasegawa(1956) described these vessels in the chicken. Wingstrand(1951) investigated them in the pigeon and other birds. Assenmacher(1952,1953, 1958) described these vessels in the duck in details.

Benoit(1962) discussed the importance of the hypothalamic control over the adeno-hypophysial gonadotrophic function through the hypothalamo-hypophysial connections in ducks. Complete involution of the gonads is obtained when the connections are divided. Assenmacher(1958) found the thyrotrophic and corticotrophic functions to be little affected after division of portal vessels or lesions of the median eminence or the hypothalamic magnocellular nuclei.

Ectopic adeno-hypophysial grafts could not activate the gonads. When the adeno-hypophysis does not get special blood supply from the median eminence, it cannot maintain normal gonadotrophic function. When the infundibular stalk is divided, complete atrophy of the posterior lobe occurs; but the gonadotrophic function is undisturbed because the median eminence, the portal system and the adeno-hypophysis remain intact. Lesion of the median eminence leads to complete and permanent atrophy of the gonads. Bilateral lesions of the anterior hypothalamic regions(supraoptic and paraventricular) is followed by genital atrophy(2-3 weeks). Testes depend completely on intact hypothalamo-hypophysial connection, the thyroids depend less and the adrenals the least. However, after section of the pituitary stalk in the rhesus monkey, Antunes *et al.*(1980) found the corticotrophs and somatotrophs to persist long after the surgery. The lactotrophs increased markedly. Gonadotrophs, although present in the pars tuberalis, were no longer demonstrable in the pars distalis 3 weeks after stalk section. The authors used immunocytochemical techniques. The pituitary stalk was sectioned transorbitally.





Kobayashi and Wada(1973) reviewed the neuroendocrinology in birds and regarding the control of gonadotrophin secretion the authors concluded, "The ventromedial region of the hypothalamus, or more precisely the nucleus tuberis, is the regulatory site of gonadotrophin secretion from the adenohypophysis. In the hen, the preoptic area is responsible for gonadotrophin release for ovulation.

Prolactin secretion in birds is regulated by the hypothalamic prolactin-releasing factor.

Regarding thyrotrophin secretion, "The anterior hypothalamus, including the nucleus paraventricularis magnocellularis and the nucleus hypothalamicus posterior medialis seems to be the site responsible for the control of thyrotrophin secretion from the adenohypophysis. Thus the regulating site is not confined to one nucleus".

Kobayashi and Wada(1973) said, "There are differences of opinion among investigators with respect to the autonomous function of cortical tissue, its dependency on adenohypophysis, and extrahypophysial corticotrophin. Nevertheless, it is obvious that cortical function is largely regulated by the hypothalamo-hypophysial system".

Gonadotrophin-releasing factor(s), prolactin-releasing factor, corticotrophin-releasing factor and growth hormone-releasing factor are present in the avian hypothalamus.

Vitums *et al.*(1964) described the vascularization of the hypothalamo-hypophysial complex in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*.

Preoptic arteries which are branches of right and left anterior cerebral arteries supply the supraoptic and paraventricular nuclei. The median eminence and the neural lobe get their supply from the infundibular artery. For the anterior and posterior divisions of the median eminence there are anterior and posterior capillary plexuses. Anterior and posterior groups of portal vessels are formed from the plexus and they are distributed to cephalic lobe and caudal lobe respectively(fig. 14.3).

Wingstrand(1951) studied the vascular supply of the avian pituitary(fig. 14.4) and in general his investigation corroborated that of Green(1951). The neural lobe has got an independent blood supply from arterial branches either from infundibular arteries or they are true inferior hypophysial arteries from inter-carotic anastomosis. The venous drainage from the neural lobe is to the sinus cavernosus. The primary plexus of the eminentia is supplied by the infundibular arteries. The drainage from this plexus is by the portal vessels contained in the portal zone of the pars tuberalis and passes to the secondary plexus in the pars distalis. The cavernous sinus drains the pars distalis.

A dense capillary net covers the median eminence on the surface or sinks deep into the furrows of the median eminence as in *Anser anser*. This capillary net extends to the surface of the infundibular stem but the density here is low.



The capillary bed of the neural lobe is independent of this system. The primary plexus is also isolated from the hypothalamic vascular bed. The hypothalamic



Fig. 14.3. Sagittal section of the hypophysis (duck). This preparation shows the right wall of the hypophysial recess (1) where the subependymal network (2) can be seen. (3), surface network lining the base of the recess, drained towards the anterior lobe (4) by the anterior portal vessels (5) and posterior portal vessels (6). (7), posterior lobe.—From Duvernoy (1972).  
 Courtesy of Professor H. Duvernoy and S. Karger AG, Basel.



capillaries are subependymal in position. The nucleus tuberis is supplied by these capillaries.

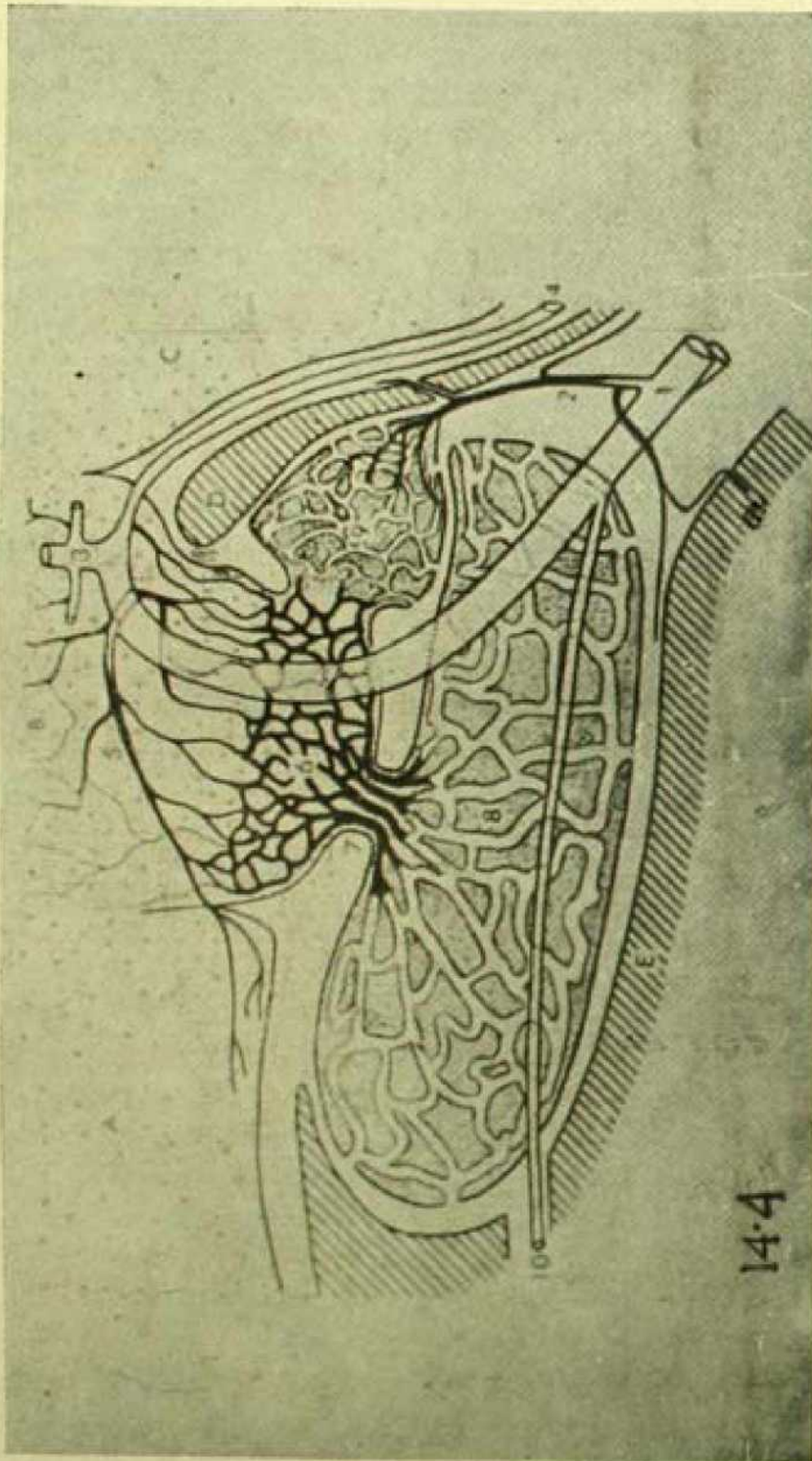


Fig. 14.4. Diagram of the vessels of the pituitary of *Columba livia*. (A), Optic chiasma; (B) Diencephalon; (C) Medulla oblongata; (D) Dorsum sellae; (E) Osseous floor of the sella.

(1) internal carotid artery; (2) Inferior hypophysial artery; (3) Anterior ramus and (4) posterior ramus of the carotid artery; (5) Infundibular artery; (6) Primary capillary plexus on median eminence; (7) Portal vessels; (8) Secondary plexus in the pars distalis; (9) Capillary bed of the neural lobe; (10) Internal ophthalmic artery.—From Wingstrand(1951).

Courtesy of Professor K. G. Wingstrand.





The blood passes from the median eminence to the pars distalis through the portal vessels and not in the opposite direction. This has been stated by Wingstrand(1951). Transport of secretory products can take place from the median eminence to the pars distalis via the portal vessels.

Szentagothai *et al.*(1968) extended the work of Torok regarding the direction of blood flow in the portal vessels of the dog, cat and rat. The pars distalis is supplied through portal vessels arising from a primary plexus, that is, plexus of the pars tuberalis and median eminence capillary loops contribute to the supply. The passage of blood is from pars tuberalis→median eminence→portal vessels→anterior lobe (pars distalis) tissue. Two other possible routes of blood flow having functional significance are present. "(i) A fraction of blood, after passing the pars tuberalis and entering the capillary loops of the median eminence, is drained over the interior median eminence plexus towards the medial(sub-ependymal) capillary network of the hypothalamus. (ii) Some of the blood, reaching the posterior surface of the anterior lobe in the pars distalis sinusoids, turns upwards and is drained over the anterior transition zone (*Umschlagszone*) between pars distalis and pars intermedia towards the posterior lobe vascular system. (iii) It is even possible that part of the blood taking route(ii) might find its way into the interior plexus of the infundibular stem and is finally drained towards the hypothalamus (i)."

Pars tuberalis is a less differentiated structure than the pars distalis but it contains some amount of trophic hormones and clearly shows signs of secretory activity (PAS-positive and Bodian-positive granules). Blood coming to the pars distalis gets a contact first with the pars tuberalis and then with the median eminence and thus the median eminence is being influenced. Some fraction of this blood reaches the median hypothalamus which is being influenced by substances produced in the pars distalis. This is a mechanism of biologically important signal transmission(Szentagothai *et al.*,1968).

The pars tuberalis of birds consists of (1) the pars tuberalis proper, which is constituted by a thin layer of cells lying within the pia mater on the surface of the brain, and (2) a portal zone having strings of epithelial cells connecting the pars tuberalis proper with the pars distalis. The portal zone is continuous with (3) the paired pars tuberalis interna which fuses intimately with the pars distalis (Wingstrand,1951). In the *Procellariiformes* the pars tuberalis has a very characteristic form and is not a vestigial organ. The pars tuberalis proper is very thick and in its central parts several layers of epithelial strings and a rich capillary net are present. The portal zone is very thick and compact.

#### *Invasion of the epithelial cells into the median eminence*

(1) The *Procellariiformes* are unique amongst birds in this respect where the tuberalis cells spread deep into the nervous tissue. This has been noted in *Diomedea melanophris*, *D. epomophora*, *Pelecanoides magellanicus*, *Priocella antarctica*, *Procellaria acquinotialis* and *Oceanites gracilis* by Wingstrand(1951).



In the Oceanites there was indication of such invasion only and this had not advanced much.

(2) Such invasion also takes place in some lizards (Gaupp, 1893; Baumgartner, 1916; Wingstrand, 1951; and Szentagothai and Szekely, 1958). Wingstrand (1951) observed this picture in *Lacerta agilis* where the epithelial anlagen of pars tuberalis after losing contact with the pars distalis reach the surface of the median eminence and enter into the nervous tissue under the pial cover. These displaced cells form small islands bilaterally. "The situation of the two intracerebral cell masses in *Lacerta* on each side of the eminentia seems to correspond to the two areas of invasion in the *Procellariiformes*" (Wingstrand, 1951).

Pars tuberalis cells with Bodian-positive granules have been found in all bird pituitaries. They are, however, rare in the tuberalis of *Columba livia* and *Anser anser* (Wingstrand, 1951). This granulation, just as the colloid acini, indicates a secretory activity of the pars tuberalis.

Cajal (1911) described the nuclei of tuber cinereum as Noyau anterieur ou principal, Noyau posterior ou accessoire du tuber cinereum, and Noyau superieur. The nucleus anterior is the same as the ventromedial nucleus. The axons of the nerve cells of this nucleus proceed in the dorsal direction and the capsule is reached; the axons proceed in anteroposterior direction. The collaterals have been very nicely described and drawn by Cajal in figs. 312, 313 and 314. The descriptions have been made in Vol. 2, pages 474 to 483. According to his description the Noyau perichiasmatic ou tangentiel has got three parts; anterior, superior and posterior. The distribution is half moon-shaped. Szentagothai *et al.* (1968) mentioned that the anterior and superior portion of the tangential nucleus of Cajal corresponds to the true neurosecretory cells of the magnocellular supraoptic nuclei. The posterior or the retrochiasmatic part of the tangential nucleus, however, has got to be differentiated from the previous group. The large cells with the whole dendritic arborization belonging to the posterior group can be beautifully stained with the Golgi and Cox methods, whereas the neurosecretory cells of the magnocellular nuclei could not be impregnated successfully. The axons of the posterior group do not join the supraopticohypophysial tract. The intercellular meshwork of preterminal collaterals is very much sparse in the supraoptic and paraventricular nuclei, but it is very rich in the retrochiasmatic part and characteristic synapses are seen here. The paraventricular nucleus has been depicted by Cajal (1911) in fig. 279, page 427 as (T) (noyau sous-ventriculaire). Szentagothai *et al.* (1968) characterized tuberal neurosecretion by argyrophilic granules and granule-laden axons could be traced to the superficial layer of the median eminence and to the proximal parts of the infundibular stem (Spatz's tubero-hypophysial system, 1951). That the fibres of hypothalamic origin could terminate in the pituitary stalk was described first by Cajal (1911) in Vol. II, page 490 of his work: "Un grand nombre des cylindres-axes destines a L'hypophyse se ramifient deja dans le pedicule et se terminent pres de sa surface par des extremités variqueuses."



Szentagothai *et al.*(1968) described the arrangement of the coarse fibred supraoptico-neurohypophysial tract and the fine-fibred tuberoinfundibular tract. The former tract originates from the large cells of the supraoptic nucleus. It is joined by fibres from the paraventricular nucleus and the conjoined fibres proceed to the posterior lobe of the pituitary. These fibres are crossed by the fine-fibred tubero-infundibular tract originating from small nerve cells situated in a halfmoonshaped area immediately beneath the walls of the third ventricle. This is the *hypophysiotrophic area*. The fine axons of the tubero-infundibular tract end exclusively in the surface zone (zona palisadica) of the median eminence and of the most proximal part of the stalk.

Isolated medial basal hypothalamus-pituitary axis, i.e., deafferented axis can operate(Halasz and Pupp,1965). Circadian rhythm of ACTH secretion is lost in chronic experiments. This axis responds by :

- (1) intermediate or high levels of nonstress pituitary-adrenal function,
- (2) stress-response to different stressors, and
- (3) low doses of dexamethasone can suppress by feedback mechanism.

Pituitary autonomy in stressed and nonstressed female rats was studied with large medial hypothalamic lesions (MHA) by noting the levels of plasma corticosterone fluorometrically by Dunn and Critchlow(1973). Hypothalamic ablation was done by modified Halasz-Pupp knife. The modification is the presence of a horizontal cross bar (3.5 mm). So, the medial basal hypothalamus is not only isolated but there is also interruption of the vascular supply. The intact vascular supply maintains the medial basal hypothalamus. Rats with medial hypothalamic lesions did not show stress(3-min. ether) response and the corticosterone levels in afternoon nonstress condition were low. A constant low level of ACTH secretion is found after ablation of the medial hypothalamus.

The extent of the lesion in the rats was from the suprachiasmatic nucleus frontally to the premammillary or mammillary nuclei caudally. Dorsally the area extended upto the paraventricular nuclei. In all animals arcuate and ventromedial nuclei were lesioned. Atrophy of the neural lobes with apparent hypertrophy of the intermediate lobes was observed. The vascularity of the median eminence and the pituitary was intact.

#### *Avian hypothalamic neurosecretory nuclei :*

These nuclear groups are mainly of two types : (a) Gomori-positive magnocellular nuclei and (b) Gomori-negative parvocellular nuclei. The supraoptic and the paraventricular nuclei include the Gomori-positive magnocellular nuclei. The infundibular and the ventromedial nuclei form the Gomori-negative parvocellular nuclei. Different names have been used by different authors for the supraoptic and paraventricular nuclei. The nucleus supraopticus corresponds to nucleus magnocellularis interstitialis, intermediolateralis, lateralis and dorsalis of Huber and Crosby(1929), nucleus magnocellularis praeopticus, medial, dorso-



caudal and lateral parts of Kurotsu(1935), nucleus supraopticus of Kuhlenbeck (1937), nucleus supraopticus of Wingstrand (1951), nucleus magnocellularis praeopticus, nucleus magnocellularis supraopticus, ventrocaudal part, medial part and lateral part of Yasuda(1955), and nucleus supraopticus, ventral, anterior, internal, external, lateral and chiasmatic groups of Legait(1959). The paraventricular nucleus corresponds to the nucleus magnocellularis interstitialis medialis (b) of Huber and Crosby(1929), nucleus magnocellularis periventricularis(Hauptkern) of Kurotsu(1935), nucleus paraventricularis magnocellularis (principal part) of Wingstrand(1951) and median and superoexternal groups of paraventricular nucleus of Legait(1959).

Gomori-positive neurosecretory cells of the *Zosterops* were divided into seven groups by Uemura and Kobayashi(1963). The nucleus supraopticus was subdivided into median and lateral groups. The nucleus paraventricularis was subdivided into anterior, periventricular and lateral groups. The sixth group was distributed in the peduncles, and the seventh group was situated in the hilar region of the median eminence. The cells of the lateral group of the nucleus supraopticus and those of the anterior and periventricular groups were stimulated when the birds were subjected to long days. Other groups were not stimulated. The neurosecretory cells of the median group of the supraoptic nucleus of the long-day birds (*Zosterops*) were activated after estrogen administration. This treatment nullified the activating effect of the long days on the neurosecretory cells of the lateral groups of the supraoptic nucleus.

Nine groups of neurosecretory cells were identified by Rossbach(1966) in the hypothalamus of the European Blackbird (*Turdus merula*). They are: nucleus entopeduncularis anterior, nucleus entopeduncularis medialis, nucleus entopeduncularis posterior, nucleus entopeduncularis ventralis, nucleus lateralis externus hypothalami, nucleus magnocellularis interstitialis dorsalis, nucleus paraventricularis dorsalis, nucleus paraventricularis ventralis, and nucleus supraopticus medialis lateralis. Seasonal activity of the gonadal cycle was reflected on the nucleus paraventricularis. Close correlation was also observed between the neurosecretory activity of the nucleus magnocellularis interstitialis dorsalis and the adrenocortical tissues.

Neurosecretory cell groups were studied by Professor Wingstrand(1951) in the pigeon and in some other birds. He has given a very beautiful description of these cells, their axons and terminations. Bluishblack granular Gomori-substance more or less completely fills the neural lobe and tends to aggregate in the glandular zones on the surface and surrounding the vessels. The tractus hypophysius in the fibre layer of the stem and the median eminence also contains these granules. Many nerve fibres look blue here but some appear red which take up phloxin used as a counterstain. No Gomori-positive substance has been noted in the caudal part of the glandular layer of the median eminence and this part is practically free from it but just behind the chiasma the glandular layer of the median eminence contains scattered Gomori-positive granules. The



tractus supraoptico-hypophysius on both sides can be traced to the nucleus supraopticus in the praeoptic region and to the scattered neurosecretory cells nearby. The neurosecretory fibres in the tractus hypophysius anterior could not, however, be traced to the scattered neurosecretory cells in the anterior part of the paraventricular nucleus.

The peripheral part of the neurosecretory cells contains dark bodies. Vacuoles of Gomori-positive substance are common in the cells.

Wingstrand(1951) stated that the nucleus supraopticus "is situated in the praeoptic area laterally of the praeoptic recess and extends laterocaudally along the dorsal surface of the tractus opticus. Scattered cells occur further back along the tractus supraoptico-hypophysius through the lateral parts of the supraoptic decussation into the anterior part of the postoptic area". This nucleus is not very distinctly delimited and its cells spread in different directions. Some cells are exclusively neurosecretory in type whereas other parts contain smaller, non-secretory cells.

Neurosecretory cells are commonly found in the periventricular cell layer near the praeoptic area but Wingstrand(1951) was uncertain whether these cells should be called as the nucleus praeopticus dorsocaudalis of Kurotsu and Kuhlenbeck or to the *principal part* of the nucleus paraventricularis magnocellularis of Kurotsu. Very few neurosecretory cells were noted in the more caudal part of the latter nucleus and the accessory part of the same nucleus did not show any sign of neurosecretion. He, however, thought of the possibility of production of other kinds of neurosecretion in the non-secretory cells.

Avian hypothalamic neurosecretory system has also been investigated by Bargmann and Jacob(1952), Benoit and Assenmacher (1953a,b), Yasuda(1955), Stutinsky(1958), Fujita(1956), Oksche *et al.*(1959, 1963, 1964), Legait(1959, Kobayashi *et al.*(1961), Arai(1963), Farner and Oksche(1962), Oksche(1962, 1965) and others. Benoit and Assenmacher(1953, 1955) and Assenmacher(1958) have extensively studied the neurosecretory system in domestic races of the mallard. Farner *et al.*(1967) have discussed the neuroendocrine mechanisms in birds.

Avian infundibular and the ventromedial nuclei are the Gomori-negative parvocellular nuclei. The infundibular nucleus controls the gonadotrophic functions.

Vasotocin producing system has been studied by Gabrion *et al.*(1978) in *Anas platyrhynchos*, *Columba columba*, and *Coturnix coturnix* using immunocytochemistry. Fluorescent reaction was noted in the anterior hypothalamic area, the median eminence and neural lobe. In the anterior hypothalamus plenty of fluorescent cell bodies were observed in the supraoptic and paraventricular regions. Fluorescent axons occupied the fibre layer of the rostral and caudal median eminence. Fluorescent fibres were also observed in the palisade layer of the rostral median eminence. Immunoreactive axons in the median



eminence contained granular inclusions of different sizes (120-160nm in diameter). These granules showed immunoreactivity. Some immunoreactive fibres containing clear vesicles (40-60nm in diameter) reached the external basal lamina. Vasotocin containing elements were distinctly different from LHRH-reactive structures. Vasotocin immunoreactivity corresponded to the aldehyde-fuchsin stainability. The authors conclude that the presence of vasotocin-containing axons in the external layer of the rostral median eminence supports the hypothesis that vasotocin is not only a posthypophysial antidiuretic hormone but also acts as an adeno-hypophysiotrophic principle at the anterior hypothalamus.

### *The median eminence and the infundibular stem*

In birds the neural lobe is always distinct and it is sharply demarcated from the infundibular stem. Diencephalic wall projects tubularly to the infundibular stem. The neural lobe has no connection with the pars distalis either by nerves or by vessels. The median eminence is the ventral or rostroventral wall of the diencephalon and extends from the optic chiasma to the infundibular stem. It is intimately related to the primary capillary plexus of hypophysiportal system (fig. 14.5). Professor Wingstrand (1951) described beautifully the structure of the median eminence, the neural lobe, the innervation, the vascular supply and the development of the avian pituitary. The external surface of the median eminence is smooth in most birds except in *Struthio* and *Anser* where it is deeply furrowed leading to a polylobed structure. In *Spheniscus* the entire wall is folded.

Three layers could be distinguished in a section through the median eminence: the stratum ependymale or ependymal layer, the stratum fibrosum or fibre layer, and the stratum glandulare or the glandular layer. Nowakowski (1951) described these three layers in the cat. The outermost glandular layer was called as palisade layer by Kobayashi *et al.* (1961). Oksche (1962) distinguished two zones: zona interna consisting of ependymal layer and fibre layer, and zona externa consisting of reticular layer and palisade layer. Aldehyde-fuchsin-positive fibres were found in the white-crowned sparrow, Zebra Finch and *C. coturnix* to leave the tractus supraoptico-hypophysius in the anterior part of the median eminence and to turn ventrally. A dense reticular formation occurs beneath the tractus supraoptico-hypophysius. Fine, radially directed fibres proceed from the reticulum towards the external limiting membrane where they are situated just opposite to and very near the primary capillaries of the hypophysial portal system. The fine fibres end in the median eminence and they do not re-enter the tractus supraoptico-hypophysius. In the posterior part of the median eminence fibres from the tractus tubero-hypophysius cross the tractus supraoptico-hypophysius. The fine fibres form loops in the zona palisadica and they do not contain aldehyde fuchsin positive material. So in the caudal part of the median eminence neurosecretory material is diminished in comparison to that noted in the cephalic division of the median eminence.



Benoit and Assenmacher(1953) noted five different layers in the median eminence of the duck. They are : (1) ependymal layer; (2) internal layer of the hypothalamo-hypophysial tract; (3) layer of fine fibres derived from 2; (4) special zone with nerve loops and neurosecretory material; and (5) pars tuberalis.

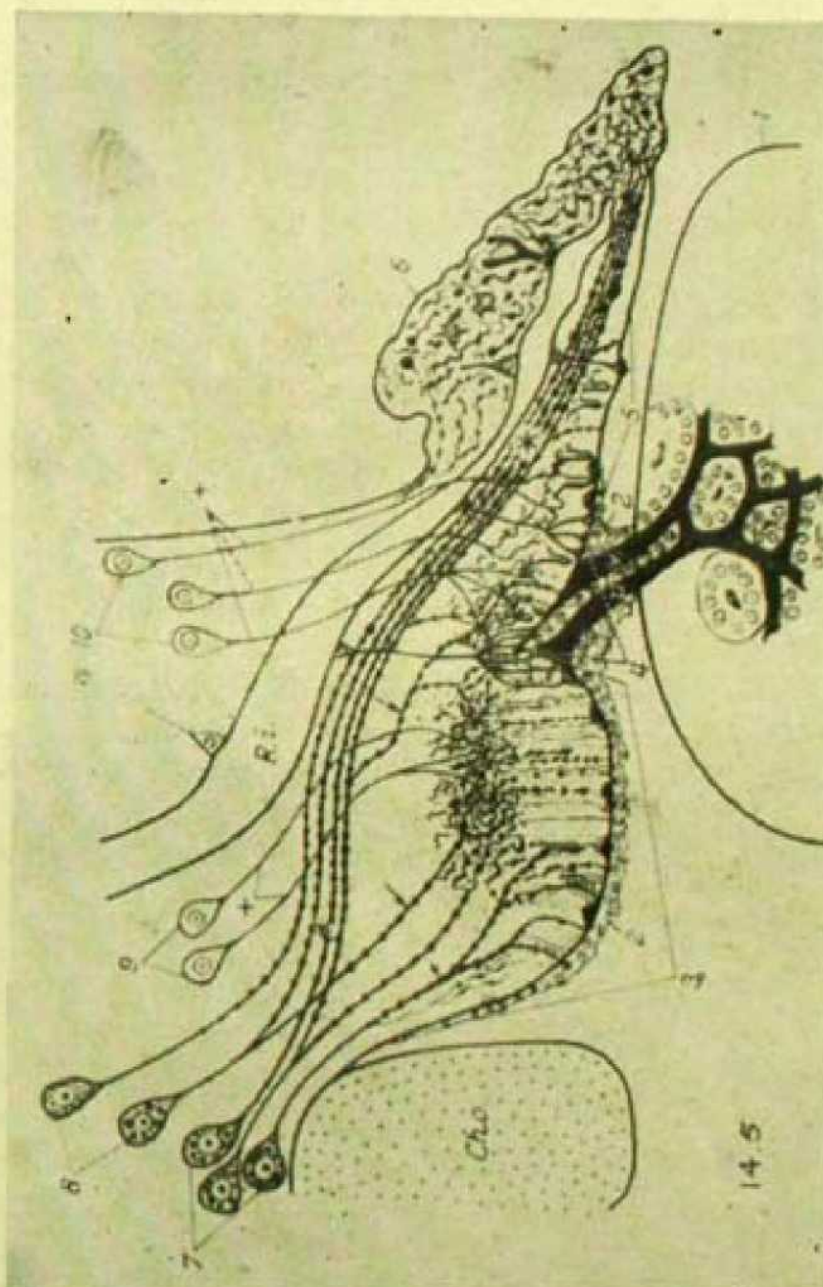


Fig. 14.5. Sagittal, lateral section through the infundibulum and the hypophysis of the White-crowned Sparrow.

(Ch. O), optic chiasma; (R.i.) infundibular recess; *Adenohypophysis* : (1), pars distalis; (2), pars tuberalis (infundibularis). *Median eminence* : (3), anterior division; (4), posterior division; (p.v.), portal vessels; (5), infundibular stem; (6), neural lobe; *Nuclei* : (7), supraoptic nucleus; (8), paraventricular nucleus; (\*), tractus supraoptico-hypophysius; (↑), beaded neurosecretory fibres penetrating into the median eminence; (9) anterior and (10) posterior-division of the infundibular nucleus; (+), tractus tuberohypophysius—From Oksche(1962).

Courtesy of Professor A. Oksche, Professor R. Diepen and Springer-Verlag, Berlin.



The ependymal layer according to Wingstrand(1951) consists of a layer of ependymal cells lining the surface of the ventricle, very few and scattered nerve fibres and a few scattered nerve cells may occasionally be present. Processes of the ependymal cells traverse through the outer two layers to reach the external surface. Flagellated or ciliated ependymal cells may be present in some birds. Single multiciliated cells were noted among the uni-flagellated ones in *Anser*. The fibre-layer is constituted by the tractus hypophysius which runs from rostral or rostrolateral directions towards the infundibular stem. Processes from ependymal cells separate the bundle of fibres one from the other. Glia cells with externally disposed processes are also noted in this layer. The glandular layer is traversed by multiple fine processes of the ependymal and glia cells. These filaments run towards the external surface and are fixed to the



Fig. 14.6. Looped nerve fibres in the external infundibular zone of the bird (after Benoit and Assenmacher) which were thought to be collaterals of the Tr. supraopticohypophysius.—From Diepen(1962). Courtesy of Professor R. Diepen and Springer-Verlag, Berlin.

intima piae by broad, dilated ends (vascular feet). The Gomori-positive substance is plenty in the fibre bundles of the tractus supraoptico-hypophysius and in the rostral part of the glandular layer but it is absent from its caudal part. Some of the coarse fibres of the tractus hypophysius can be seen in the glandular layer of the median eminence only in its rostral part. Fine, looped nerve fibres are noted in this zone parallel to the ependymal fibres (fig. 14.6).



### The neurohypophysis

The neurosecretory substance produced in the supraoptic and paraventricular nuclei of the birds is accumulated in the median eminence and the neurohypophysis. There are four different types of neural lobes in birds.

Type I is noted in *Strigiformes*, *Procellariiformes*, and *Galliformes*. In these orders the neural lobe is very simple. It is formed by hollow buds with thin primitive walls. Emigrated secondary tissue from the primitive infundibular one is very less. Only ependymal cells are present and pituicytes are rare. In adults the paired condition established in young embryos by the development of primary branches is hardly visible because it is hidden by the numerous hollow buds. Highly complicated neural lobes are found in *Procellariiformes* and *Galliformes*. *Perdix perdix* shows compact type of neural lobe.

Type II: This type is noted in majority of birds including *Struthio*, *Oceanites*, *Nycticorax*, *Charadriiformes*, *Columbiformes*, *Falconiformes*, *Psittaciformes*, *Piciformes*, *Apodiformes*, and *Passeriformes*. There is a central lumen near the base of the lobe and this lumen extends bilaterally as blind diverticula and each of which may sometimes divide into two or three diverticula. The walls are thick and secondary tissue forms most of the gland.

Type III: This type is found in *Pelecanoides*, *Priocella*, *Phalacrocorax*, *Anseriformes*, *Larus*, *Perdix*, *Cuculus*, and *Caprimulgus*. The neural lobe consists of more of secondary tissue and so the organ has a compact appearance. The lumen is found only at the base of the gland. On the rostro-lateral surface a pair of lateral primary diverticula are found. One or a few median diverticula may also be found. The pituicytes are plenty except in *Perdix* where the cells are few. The ependymal cells are distributed in a limited fashion.

Type IV: This type is seen in *Spheniscus*. The neural lobe is compact. Lobular character is lost. There are scattered membranes and connective tissue fibres within the gland. Narrow channels lined by ependyma are found in all parts of the gland. Ependymal cells and pituicytes are of equal number. Budding from the primitive sac is plenty but the buds lose their superficial membranes because of fusion. Only the connective tissue fibres are present.

The ependymal cells in the neural lobe are like those noted in the median eminence but with the difference that here the processes do not branch so much. The ependymal processes get attachment to the superficial membrane of the primitive walls or to the membrane around vessels in the secondary tissue (types III and IV) by conical vascular feet. The pituicyte has the shape of an irregular star with 2-4 processes and they are attached to the connective tissue fragments and to the intima piae around capillaries in the secondary tissue.



*Nerve fibres to the median eminence and the neural lobe of the pigeon*

\* The description has been taken from Wingstrand(1951). All the nerve fibres at the junction between the neural lobe and the stem are concentrated in a tube-shaped tract surrounding the lumen of the stem. Towards the eminential side the tubeshaped tract continues in the fibre layer of the eminentia. The fibre path is known as tractus hypophysius and because it can be traced to the nucleus supraopticus it is called tractus supraoptico-hypophysius. The tractus hypophysius posterior is formed by few coarse fibres which separate from the tractus hypophysius just at the base of the stem and turn upwards along the posterior surface of the hypothalamus. Superficial eminential plexus is constituted outside the fibre layer in the median eminence and in the proximal parts of the stem by a diffuse plexus of delicate and irregular fibres. This plexus is mainly formed by tractus tubero-hypophysius coming from the tuber nuclei. Numerous fibres with an irregular course end in this plexus and there are also transverse fibres and decussation. This plexus innervates the glandular layer. The peculiar loops in the glandular layer take their origin exclusively from this plexus. The course of the tractus tubero-hypophysius before joining the superficial eminential plexus is very peculiar. From the tuber nuclei some fibres proceed along the lateral surfaces of the hypothalamus and enter the eminentia close to the intima pia and situated outside the tractus hypophysius. Some other fibres take a separate course along the ventricle and reach the ependymal layer inside the tractus hypophysius. These fibres run ventromedially and then laterally passing between the bundles of the tractus perpendicular to the surface and join the superficial plexus. In the anterior third of the eminentia superficial plexus is still present and it can not be separately distinguished from the fibre layer as the two lie very close to each other and the glandular layer receives some fibres from the tractus hypophysius and these fibres form the peculiar loops in the glandular layer. The superficial plexus in this part gets also plenty of fibres from tubero-hypophysial tract. Postchiasmatic crossed and uncrossed fibres form the tractus supraoptico-hypophysius. The tractus hypophysius anterior is formed by all postchiasmatic hypophysial fibres which do not belong to the tractus supraoptico-hypophysius or the tractus tubero-hypophysius.

The tractus hypophysius posterior arises from a diffuse nucleus situated ventral to the decussatio tractus infundibuli and is called nucleus subdecussationis by Wingstrand(1951). The tractus tubero-hypophysius is formed by fibres coming from the tuberal nucleus which is same as Kuhlenbeck's nucleus tuberis and Huber and Crosby's nucleus hypothalamicus inferior + nucleus mamillaris medialis ventralis and Rendahl's *nucleus m.* The tractus supraoptico-hypophysius arises from the supraoptic nucleus. The fibres of the tractus hypophysius anterior can be traced to the lateral and inferior hypothalamic nuclei, and Kuhlenbeck's *n.paraventricularis posterior hypothalami*, *n.paraventricularis magnocellularis* and *n.praeopticus paraventricularis*.



*Electron microscopic structure of the avian median eminence and the neural lobe*

Kobayashi *et al.*(1961) examined the median eminence of the parakeet, *Melopsittacus undulatus* and noted the processes of ependymal cells, neuroglial cells and terminal parts of axons with four types of structures. The processes of ependymal cells could be identified by the presence of large number of fibrillar structures. At the terminal segments of axon paths there were structures with average diameters of 390Å (synaptic vesicles), other larger ovoid vesicles with diameters of 490Å, elementary neurosecretory granules (600 to 1000Å) and vesicles of the same size as the elementary granules. Structures which are intermediate between synaptic vesicles and ovoid vesicles could be found. Presence of all or some of these structures could be found in different nerve terminations. Folds of thick basement membrane of the primary capillaries of the hypophysial portal system enter into the median eminence and axon endings with synaptic and larger vesicles terminate on the membrane. Processes of ependymal and neuroglial cells interpose between the nerve terminations and the basement membrane. Typical elementary granules and vesicular structures are found in the fibre layer of the median eminence. Processes of ependymal fibres and sections of nerves have also been noted. Oota and Kobayashi(1962) and Bern and Nishioka(1965) studied the ultrastructure of the avian median eminence.

Duncan(1956), Legait and Legait(1958), and Kobayashi *et al.*(1961) noted the terminations of the neurosecretory axons in the neural lobe. These terminations are surrounded by pituicytic cytoplasmic processes. The terminations contained the same structures noted in the median eminence. The granules vary in size from 600 to 1000 Å in the terminations located in the median eminence but the diameter ranges from 600 to 1750 Å in the neural lobe.

Oksche(1965), Oehmke *et al.*(1969) and Oksche *et al.*(1970) studied the hypothalamo-hypophysial system of birds with the help of electron microscope. They found in *Passer domesticus* that the granules (diameters up to 1000 Å) of the tubero-infundibular tract are formed in the Golgi zone of the cells of the infundibular nucleus. Axodendritic and axosomatic synapses are found in the nerve cells of the infundibular nucleus. Different types of structures are noted in the nerve endings of median eminence. They contain vesicles (400-600Å), vesicles (400Å) and granules (800Å), granules (800-1000Å), vesicles (300-400Å) and granules (1200Å) and granules from 1200Å to 1600Å. In the pars nervosa of the hypophysis the diameter of the granules is from 2000 Å to 2500 Å.

Farner *et al.*(1967) in their survey on neuroendocrine mechanisms in birds said, "A very important question is that of possible functional synaptic contacts between neurosecretory and Gomori-negative fibres either in the reticular layer or in the peripheral part of the palisade layer." This type of functional relationship was raised in the past by many authors.



Calas and Assenmacher(1970) studied the ultrastructure of median eminence in the canard (*Anas platyrhynchos*). Within the subependymal layer neurons of the nucleus infundibularis, axons and varicosities with dense core granules, and capillaries with structural characteristics similar to those in the superficial layer were present. The axons of the hypothalamo-neurohypophysial tract were seen in the fibre layer. Evidence for at least three main types of neurosecretory axons are seen in the palisade layer. Peczely and Calas(1970) described the ultrastructure of the median eminence in the pigeon (*Columba livia domestica*) in different experimental conditions. Hypothalamic control of adrenocorticotrophic function was specially discussed with particular reference to the role of the 1200-1400 Å granules

*Enzymatic activity in the median eminence and the neural lobe*

Kobayashi *et al.*(1962) found increase in acid phosphatase activity in the neural lobe of dehydrated pigeons without any increased activity in the median eminence. Similar feature was noted in the Whitecrowned sparrow by Farner *et al.*(1964) and Kawashima *et al.*(1964). Similarly, increased acid phosphatase activity has been noted in the median eminence without any change in the neural lobe after photostimulation of testicular growth in the white-crowned sparrow by Kobayashi and Farner(1960), Farner *et al.*(1964) and in *Zonotrichia albicollis* by Wolfson and Kobayashi(1962). This proves a dissociated response in between the median eminence and the neural lobe and that an acid phosphatase may be responsible for the release of neurohormones into the primary capillaries of hypophysiportal vessels.

Photoperiodic gonadal stimulation in *Zonotrichia leucophrys gambelii* (the White-crowned sparrow) led to an increase in catheptic proteinase activity in the median eminence, whereas Kobayashi *et al.*(1962) did not find any such change in the neural lobe. This is also an example of the dissociated response; but dehydration increases such activity in the neural lobe and the median eminence. Increased activity in the neural lobe is associated with the increased release of antidiuretic hormone. Increased activity at the median eminence is due to the concomitant release of corticotrophin-releasing factor (CRF) as Kawashima *et al.*(1964) could find that there was depletion of sudanophilic material in the interrenal cells after dehydration.

Acetylcholinesterase activity was found in the median eminence of the White-crowned sparrow by Kobayashi and Farner(1964). Uemura(1964) noted this activity in *Zosterops palpebrosa japonica*. Kobayashi(1965) found it in *Passer montanus*. AChE activity is associated with aldehyde-fuchsin-positive and aldehyde-fuchsin-negative endings. The activity is weaker in the neural lobe. Cholinergic mechanisms may help in the release of releasing factors.

Monoamine oxidase (MAO) activity in the neurosecretory system of the tree sparrow was studied by Matsui(1964). Moderate MAO activity was seen in the AF-positive neurosecretory cells. Reaction in the pars nervosa was slight.



MAO activity was noted in the median eminence surrounding the small blood vessels or primary capillaries of the hypophysiportal system. Little or no reaction was found in the supraoptico-hypophysial tract and the ependymal cells. The cytoplasm of the glia cells gave a strong reaction. MAO activity in the median eminence is located mainly in the aldehyde-fuchsin-negative nerve endings and/or the processes of the glia cells. As catecholamines are not present in the glia cells, MAO is present only at the terminations of the aldehyde-fuchsin-negative fibres of the median eminence (posterior part). Thus adrenergic mechanisms may help in the release of releasing factors into the primary capillaries of the hypophysiportal system of the median eminence.

### *Avian hypothalamic monoamines*

Avian hypothalamic monoamines have been demonstrated by Fuxe and Ljunggren(1965), Bjorklund *et al.*(1968), Sharp and Follett(1968), Oehmke *et al.*(1969), Sharp and Follett(1970), Oksche *et al.*(1970) and Oksche(1971) with histochemical fluorescent technique.

Fuxe and Ljunggren(1965) studied the cellular localization of monoamines in the upper brain stem of the pigeon. Green and yellow fluorescence developed indicating the presence of a primary catecholamine and 5-hydroxytryptamine respectively. Low concentration of amines is found in the cell bodies and axons but very high concentration is found in the terminals, specially in the abundant varicosities. Three large ascending monoamine systems are present within the upper brain stem. The fibres start from cell bodies located within the mesencephalon and run mainly in the medial forebrain bundle. Two of the neuron systems produce and store a primary catecholamine, one probably giving rise mainly to terminals within the hypothalamus and the praeoptic area, the other within the corpus striatum. The remaining system produces and stores 5-hydroxytryptamine.

In the median eminence (palisade layer) fluorescent terminals are small in number though they are variable. Sharp and Follett(1970) stated that the fluorescent terminals in the palisade layer are probably derived from the monoamine containing axons in the subependymal layer. Bjorklund *et al.*(1968) found stronger fluorescence in the chicken than in the pigeon. Sharp and Follett(1968) observed similar terminals in the anterior and posterior divisions of the quail median eminence. The terminals end on the primary capillary plexus. Sharp and Follett(1970) stated that in the reticular layer fluorescent structures are few but there are some coarse beaded fibres which run from cephalic to caudal direction.

Fuorescent nerve terminals were noted in the nucleus tuberis and the nucleus hypothalamicus posterior medialis. Sharp and Follett(1970) found catecholamine-containing nerves to proceed from nucleus tuberis to the subependymal layer after coursing around the base of the third ventricle and so



this forms a part of the tubero-hypophysial tract in the quail. Fibres from the nucleus hypothalamicus posterior medialis may also add to the previous fibre system.

Fluorescent cell bodies have not yet been well detected in the avian hypothalamus although in the mammalian nucleus tuberis catecholamine containing cell bodies are seen well in pregnancy and lactation.

The nucleus hypothalamicus posterior medialis is placed in such a central location for reception, modulation or transmission of information that message can easily be conveyed by adrenergic pathways between higher brain centres, anterior hypothalamus or lower brain stem and the nucleus tuberis or median eminence. Sharp and Follett(1970) found fluorescent tracts to proceed in the stratum cellulare internum connecting with the anterior hypothalamus while lateral tracts join the forebrain bundle.

In the anterior hypothalamus the supraoptic and paraventricular nuclei are surrounded by monoaminergic nerve fibres. Plexus of adrenergic fibres is more in the medial division of the supraoptic nucleus than in the lateral division. The adrenergic fibres of this system come from cells located in the pons and medulla.

Dopaminergic neural system starting in the hindbrain and terminating in the nucleus basalis and the forebrain after passing through the forebrain bundle also occurs in the avian brain.

Monoaminergic supraopticohypophysial system also occurs in birds. The fibres come from the medial division of the supraoptic nucleus.

Oksche(1971) summarized the observations regarding detection and localization of avian neurons producing neurohormones and releasers with special reference to the hypothalamo-hypophysial system. The anterior division of the median eminence is Gomori-positive. The same positive reaction is noted in the supraoptic and paraventricular nuclei and in the neurosecretory pathway leading to the neural lobe. With Falc-Hillarp fluorescence preparation, arcuate (infundibular) nucleus with fluorescent structures is seen but the neural lobe gives a complete negative picture. Amongst and in between fluorescent and nonfluorescent structures of the hypothalamus there are some other types of cells which should be considered as releasers. In the White-crowned sparrow the anterior group of portal vessels is connected with the anterior median eminence and the cephalic lobe of the anterior pituitary and posterior group connects the posterior median eminence with the caudal lobe. The anterior median eminence is Gomori-positive whereas the posterior median eminence is Gomori-negative. Lesion near the optic chiasma interrupts the supply of Gomori-positive substance to the anterior median eminence. Bern and Nishioka(1965) noted in the house sparrow that the elementary granules in the neural lobe are bigger than those noted in Gomori-positive area. Oksche(1967) and Oehmke *et al.*(1969)



observed in the house sparrow that the supraoptico-and paraventriculo-hypophyseal tracts contain the elementary granules with a diameter of 200 nm but the largest granules have got a diameter of 120-150 nm in the anterior median eminence. He suggested that the anterior hypothalamus contains the cells wherefrom these aldehyde-fuchsin-positive fibres come to the anterior median eminence. Amongst the cells of the supraoptic nucleus having 200 nm granules some cells with smaller (120-150 nm) granules were observed. These correspond to those smaller granules noted in the anterior median eminence (Gomori-positive endings). He further said, "There are also some fluorescent elements in the proximal neurosecretory pathway, so that this part of the tract does not contain only nerve fibres connecting the anterior hypothalamus with the neural lobe but also other fibres, fluorescent or non-fluorescent, connecting some anterior nuclei of the hypothalamus with the ME".

Boissin and Assenmacher(1971) discussed the implication of the central aminergic mechanisms regarding the determination of the circadian rhythm of blood corticosterone in the quail.

Peczley(1971) studied the effect of metyrapone, prednisolone and insulin treatment on the domestic pigeon's hypothalamus. Close functional correlation exists between the aldehyde-fuchsin-positive neurosecretory system of the anterior hypothalamus and the median eminence and the corticotrophic activity. The arcuate nucleus may play an important part in the regulation of ACTH secretion.

Bouille and Bayle(1973) conducted experimental studies on the adrenocorticotrophic area in pigeon hypothalamus. In the posterior medial and lateral hypothalamic areas there is a well-defined adrenocorticotrophic area, destruction of which leads to the same decrease of the plasma corticosterone as is noted after adeno-hypophysectomy and this also prevents the progressive recovery of adrenocortical function after autografting.

Calas(1973) studied the monoaminergic innervation of the median eminence in the canard (duck) *Anas platyrhynchos* including radioautographic and pharmacologic studies. Findings suggest that the neurons of the median eminence are probably modulated by catecholamines and other neurotransmitters on their dendrites and on their soma.

By an autoradiographic study of sex steroids in the chicken telencephalon Wood-Gush *et al.*(1977) found estrogen, progesterone, and testosterone labeled cells in the hyperstriatum ventrale. The general distribution was similar with each hormone. Estrogen and testosterone were more extensively distributed. Progesterone was confined to a small area of the lateral hyperstriatum. Estrogen and testosterone also labeled cells in the hyperstriatum dorsale and in the nucleus intercalatus hyperstriaticus.

Cells in the male fowl brain accumulating radioactivity after  $^3\text{H}$  testosterone administration were identified by autoradiography (Barfield *et al.*,1978). Labeled



cells were mainly found in hypothalamic, limbic and midbrain structures. This was comparable to that for sex hormone uptake in vertebrates in general. Areas concerned with sex hormone-dependent functions generally accumulated T. Marked uptake was noted in the preoptic area, in the anterior and posterior hypothalamus, in the archistriatum particularly in the nucleus taeniae, and in the lateral septum. In the midbrain, substantial uptake of labeled hormone was noted in the nucleus intercollicularis.

Hypothalamic lesions were produced in White Leghorn hens by Egge *et al.* (1975). They include lesions of *septomesencephalic tract* (tract lesions) and the *supraoptic or ventrolateral nuclei* (nuclear lesions). With tract lesions rostral lobes of the pituitary had higher levels of TSH than noted in controls. There was no change in the caudal lobe. When lesions were produced in the nuclei, an insignificant decrease in TSH levels in the rostral lobe was observed. Insignificant increase in the caudal lobe was noted with nuclear lesions. Radke and Chiasson (1977) concluded that thyrotrophs responding to circulating levels of thyroid hormone were located throughout both the lobes of the gland but thyrotrophs responding to hypothalamic TRH were only situated in the rostral lobe.

#### *Cell types of the avian pars distalis*

The cell types have been described by Rahn and Painter (1941), Wings-trand (1951, 1963), Mikami (1958, 1960), Tixier-Vidal *et al.* (1962), Benoit (1962) and Tixier-Vidal (1963). The avian adenohypophysis has no intermedia. The pars tuberalis is constantly present and the pars distalis is divided into a cephalic lobe and a caudal lobe.

Benoit (1962) described the histology of the duck's pituitary. Acidophilic cells are alpha and eta and the glycoproteidic cells are beta, gamma, and delta cells. There are three types of gonadotrophic cells. Gamma cell which is one type of gonadotrophic cell is found only in the caudal lobe of the pituitary. It is carminophilic in Azan stain, purple in Herlant's tetrachrome, light blue in Methasol blue PAS method and it is PAS-positive. Period of activity is from December to April (testicular growth period). The cells involute with regression of testes. These cells produce LH.

Cephalic lobe beta cells are not carminophilic. They are PAS positive and purple with Herlant's dye. With Methasol blue-PAS these cells look violet blue. These cells are active from February to July. They secrete FSH. The eta cells are found in the cephalic lobe. They are erythrosinophilic in Herlant's dye, PAS negative, and look light blue with Methasol blue-PAS. They are active from December to April. Probably they secrete LTH.

Seven cell types have been demonstrated in the anterior hypophysis of the duck by Tixier-Vidal (1963). They are the three mucoprotein forms (beta,



delta and gamma) and three acidophilic forms : (alpha, eta and epsilon). The seventh type called kappa is peculiar to birds. Alpha and gamma cells are found in the caudal lobe and the beta, eta, epsilon and kappa cells are located in the cephalic lobe. The delta cells are equidistributed between the two lobes. Identification of FSH-beta cells, LH-gamma cells, prolactin-eta cells and TSH-delta cells could be done. Corticotrophic activity seemed to be localized in the epsilon acidophilic cells. STH could be secreted by alpha cells and kappa cells are the source of MSH.

The cells of the cephalic lobe are controlled primarily by neurohormones secreted by supraoptic and paraventricular nuclei and the cells of the caudal lobe are controlled by neurohormones from the infundibular nuclei (Farner *et al.*, 1967).

*Brain mechanisms responsible for ACTH release in the pigeon  
(Columba livia)*

de Roos(1963) reviewed the physiology of the avian interrenal gland. It is stated that corticosterone and aldosterone are the major corticoid hormones secreted by the avian interrenal. The interrenal is dependent on the anterior pituitary for normal function but there is a great deal of controversy regarding the degree of such control. Even in the absence of the pituitary, the interrenal functions at a high level (Miller and Riddle,1942; Miller,1961; Assenmacher,1958; Benoit,1962; Ma and Nalbandov,1963). Farner *et al.*(1967) concluded that the avian adrenocorticotrophic activity of the pars distalis is only partially regulated by hypothalamic neuroendocrine mechanisms. Semi-independent function of the adrenal cortex is possible in the absence of the pars distalis. Peczely(1971) and Bouille and Bayle(1973) indicated the existence of hypothalamic adrenocorticotrophic area in the pigeon. Salem *et al.*(1970) had indications that chicken hypothalami contain ACTH or an ACTH-like substance. They also indicated that the adrenal ascorbic acid depletion in hypophysectomized rats by chicken hypothalamic extracts was not due to vasotocin. The hypothalamic depleting substance was different from hypophysial ACTH. Corticotrophin releasing factor (CRF) is present in the chicken hypothalami.

Bouille and Bayle(1973/74) studied the effects of limbic stimulations or lesions on basal and stress-induced hypothalamic-pituitary-adrenocortical activity in the pigeon. A marked drop or rise in plasma corticosterone concentration was observed after hippocampal stimulations or lesions. Corticosterone(B) titer increased after archistriate stimulation. Hippocampal lesions suppressed diurnal variations in corticoid level. However, this lesion only slightly reduced the stress-induced responses. No behavioral signs were detected during and after periods of stimulation.



Comparison between hypothalamic, hippocampal and septal multiple unit activity (MUA) and basal corticotrophic function in unrestrained, unanesthetized resting pigeons was made by Bouille and Bayle(1976). "The pattern of electrical activity recorded from the adrenocorticotrophic area of the hypothalamus showed diurnal variations which paralleled the plasma corticosterone fluctuations during 24h photoperiod. Both parameters were low in the late afternoon and the evening and high in the early morning. Hypothalamic activation slightly preceded the peak of corticosteronemia. Conversely, in hippocampal(H) and septal(S) regions, the peak of MUA occurred in phase opposition with respect to the hypothalamic peak, and there was a marked decrease of firing rates at the moment when adrenocorticotrophic activation was initiated.

Comparison was made between hypothalamic multiple-unit activity and corticotrophic function after bilateral destruction of the hippocampus of the pigeons by Bouille and Bayle(1978). In unrestrained resting pigeons multiple unit activity (MUA) was recorded from the adrenocorticotrophic area (n. posterior medialis hypothalami, PMH) throughout the 24h period and compared with plasma corticosterone(B) levels. Diurnal variations of MUA and B were found to be suppressed after bilateral electrolytic lesions of the hippocampus. "Both parameters were stabilized at a steady high level whereas complete neural isolation of the basal hypothalamus led to stabilized intermediate plasma B level and MUA pattern."

Ramade *et al.*(1980) studied adrenocortical responses to systemic or neurogenic stress and to hypothalamic stimulation in chronically catheterized thalamic pigeons (*Columba livia*). They suggested, "Extrahypothalamic neuronal networks are responsible for the long-lasting repetitive adrenocorticotrophic response to stress, which are not involved in the single response to hypothalamic stimulation itself. Furthermore, such extrahypothalamic neuronal networks should be located at the diencephalic or rhombencephalic level since hemispherectomized pigeons exhibited the same profile of stress-induced episodic hypercorticosteronemia as seen in intact birds."

#### *Observations (From Roy,1976)*

- (1) Feedback receptor sites in the median eminence region of the pigeon sensitive to adrenocortical steroids :

Corticosterone, cortisol and dexamethasone have been implanted into the basal region of the hypothalamus (median eminence), dorsal hypothalamus and pituitary. The animals were killed by decapitation one week after operation. Adrenal weight, plasma and adrenal corticosterone levels were analysed. Diminished adrenal weight, and diminution of plasma and adrenal corticosterone



levels were found when dexamethasone was put in the median eminence. Cortisol and Corticosterone implanted into the median eminence had similar results except the fact that they could not change the adrenal weight. Dorsal hypothalamic and anterior pituitary implantation had no effect. The responses were more in the dexamethasone group.

50% fall of plasma corticosterone level occurred when ACTH was implanted into the median eminence without any change in the adrenal weight whatsoever (three days after the implantation).

(2) Corticotrophin releasing factor (CRF) after destruction of supraoptic, paraventricular, tuberal and ventromedial nuclei and anterior median eminence of the pigeon :

These lesions (electrolytic) when singly done could not reduce the CRF of the median eminence; rather it was high on some occasions. It proves thereby that CRF producing neurons are not localised to any of the particular areas concerned. Maximum increase was observed in the group where the anterior median eminence was damaged. Plasma corticosterone levels were measured after intracarotid injection of the hypothalamic extracts [Prepared after the method of Vernikos-Danellis(1964)] in dexamethasone blocked (median eminence) pigeons.

(3) CRF in the median eminence and plasma of the hypophysiportal vessels of the pigeon :

CRF has been found in the median eminence. There is a rise in the CRF content after the stress of ether anaesthesia for 45 minutes or fracture (1 day). The hypophysiportal vessels have been approached through the transorbital route and divided. Blood collection rate varied between 0.4 ml. and 0.5 ml./hour. Plasma corticosterone levels were measured after intracarotid injection of the portal vessel plasma in dexamethasone blocked (median eminence) pigeons. Presence of CRF in the portal vessel plasma has been noted in the pigeon. CRF in the pigeons' portal vessel plasma diminished in animals with implantation of corticosterone, cortisol and dexamethasone in the basal region of the hypothalamus (median eminence). Plasma from peripheral vessels did not contain CRF activity to any great extent as compared to the results obtained by injection of portal plasma.

(4) Aldehyde-fuchsin-positive material and plasma corticosterone level in normal and stressed (fracture) pigeons :



## Aldehyde-fuchsin-positive material

	Supraoptic nucleus.	Para- ventri- cular nucleus.	Anterior median eminence.	Posterior median eminence.	Neural lobe.	Rise in plasma corticos- terone level.
Normal pigeon (5)	+	+	+	O	+	
Stress (fracture of right femur) (3rd Hr.) (7)	Partial depletion in some animals.	O	Partial depletion & not detected in all animals.		O	91%
Stress (fracture of right femur) (1 day) (8)	Depletion in more number of animals.	O	Depletion in more number of animals.	O	O	100%

+ = Present ; O = No change ; ( ) = Number of animals.

After the method of Guillemin *et al.* (1959), modification of Silber *et al.* (1958).

(5) Changes in epsilon cells of the anterior pituitary, and adrenal in response to fracture and dexamethasone injection in the pigeon :

Types of treatment.	Epsilon cells (ACTH producing cells)	Adrenal
Normal	These cells are located in the cephalic lobe of the pituitary and start from the junction of cephalic and caudal lobe and spread throughout the cephalic lobe. Herlant's tetrachrome stains them purple rose. These are small round cells. Sometimes the granulations are very faint and vacuolations occur. The nucleus has got a nucleolus.	Functional zonation is possible. The subcapsular zone produces aldosterone. The deeper layers produce corticosterone and are controlled by ACTH.
Fracture of right femur. (5 to 7 days).	There was increased number of these cells. The granulations could easily be detected.	Stimulation.
Dexamethasone (1 mg./day for 6 days).	The activity of these cells was below normal and they were less in number and atrophic.	Atrophic conditions.





## (6) Adenohypophysial grafting in the medial basal hypothalamus of the pigeon and other areas: cellular morphology.

Location of the graft	Cellular morphology		Cellular morphology) Stress response to fracture (3-5 days).
	Herlant's tetrachrome	Alcian blue-PAS-orange G	
(1) Upper eyelid	Cells were small in size. There was diminished cytoplasm and the nuclei were also small.		No change could be seen.
(2) Dorsal hypothalamus (basal aspect)			
(3) Ventral hypothalamus (median eminence, tuberal nucleus, ventromedial nucleus and retrochiasmatic area adjoining the aldehyde-fuchsin-positive fibre-area)	Active granulated cell types could be seen. PAS-positive basophils and blue delta basophils could be identified. The grafts also contained eta and epsilon cells.		Increased activity in the epsilon cells and the basophils could be observed.

## (7) Stimulation and lesion experiments of the brain in the pigeon and ACTH release:

Freely moving birds, with chronically implanted electrodes of 0.3 mm. in diameter with insulation except at the tip were used for stimulation experiments. These were bilaterally implanted either superficially or at a depth. In the control series no stimulation was applied. The frequency of stimulation was 15-30 cps, the duration of the rectangular pulse was 3 msec, the voltage used was from 3 to 5 V. The total time of stimulation varied from 3 to 8 minutes.

In the lesion experiments, surface lesions were produced surgically and depth-lesions were produced by electrocautery.

Peripheral blood corticosterone levels were studied  $\frac{1}{2}$  hour after stimulation and in the lesion experiments after 3 to 5 days.

Stimulation experiments	Release of ACTH
(1) Ventral and posterior hypothalamic stimulation- median eminence, nucleus hypothalamicus posteromedialis, nucleus inferior and nucleus arcuatus.	Increased ACTH release.
(2) Archistriatal stimulation.	Increased ACTH release
(3) Hippocampal, septal and septomesencephalic tract.	Diminished ACTH release

Lesion experiments	ACTH Release	Stress of fracture and ACTH release.
Hippocampus, septum, septomesencephalic tract and medial forebrain bundle.	Increased ACTH release	Increased ACTH release.



### *Summary and conclusion*

- (1) There are feedback receptor sites in the median eminence region of the pigeon (*Columba livia*) which are sensitive to adrenocortical steroids.
- (2) Corticotrophin releasing factor (CRF) is present in the median eminence. CRF producing neurons are not localized to any particular ventral hypothalamic nuclear groups, rather a widespread nuclear chain is involved in this process.
- (3) Stress of ether anaesthesia or fracture leads to a rise in CRF content of the median eminence. The hypophysiportal vessels plasma contains CRF.
- (4) Evidences of correlation have been observed between the aldehyde-fuchsin-positive material in the supraoptic nucleus, anterior median eminence and plasma corticosterone level. Stress responses have been observed.
- (5) Epsilon cells in the cephalic lobe of the pars distalis of *Columba livia* produce and secrete ACTH. Stress of fracture stimulates the pituitary-adrenal-axis and dexamethasone inhibits it.
- (6) Hypophysiotrophic area (median eminence, tuberal nucleus, ventromedial nucleus and retrochiasmatic area adjoining the aldehyde-fuchsin-positive area) could be identified in *Columba livia* from pituitary grafting (in medial basal hypothalamus) experiments. Stress response could be observed in the epsilon cells and basophils of the grafted pituitaries.
- (7) Stimulation and lesion experiments of the brain of *Columba livia* have been conducted with a view to note the control of ACTH release. Areas from where increased ACTH release has been obtained on stimulation are ventral and posterior hypothalamus (median eminence, nucleus hypothalamicus postero-medialis, nucleus inferior and arcuate nucleus), and archistriatum. Hippocampus, septum, septomesencephalic tract and medial forebrain bundle are inhibitory areas for ACTH release.

### *Further reviews on neuroendocrinology in birds*

This subject has been reviewed by Kobayashi and Wada(1973) in greater details.

According to Kobayashi (from Kobayashi *et al.*, 1970) the median eminence on its external surface is covered by the capillaries of the primary plexus of the hypophysial portal veins and interiorly the basal portion of the hypothalamus occupied by the processes of the secretory ependymal cells. (There are some cells of the infundibular nucleus in this portion). Kobayashi and Wada(1973) tabulated the structural components of the median eminence of birds and noticed an internal and an external zone. The internal zone had three layers. The ependymal layer had ependymal cells. Hypendymal layer had hypendymal cells and glial cells. The fibre layer consisted of ependymal processes, supraopticohypophysial tract, glial cells, ependymal, hypendymal and glial processes. The external zone consisted of two layers : reticular layer and palisade layer. The components of the



reticular layer are supraopticohypophysial tract, tubero-or infundibulo-hypophysial tract, glial cells, ependymal, hypendymal and glial processes. The components of the palisade layer were the same as those of reticular layer; but after leaving the reticular layer, all the fibres and processes proceed in palisade fashion to the basal surface of the median eminence in this layer.

The authors further stated that some AF positive fibres starting from the nucleus supraopticus and nucleus paraventricularis proceed in the fibre layer towards the pars nervosa as supraopticohypophysial tract. In the anterior median eminence, other AF positive fibres descend to the palisade layer. AF negative fibres end in both the anterior and posterior median eminences and this has been detected ultrastructurally. The terminations are near the capillaries of the primary plexus of the hypophysial portal vessels. Large granules (1500-2000 Å) may be carriers of neurohypophysial hormones and granules of 1000 Å may be the carriers of monoamines. In the anterior median eminence the large granules are plenty; but they are rare in the posterior median eminence. In the pigeon large granules increased in number in the posterior median eminence after hypophysectomy. In both the anterior and the posterior median eminences granules of 1000 Å are present. Other granules may carry possibly releasing factors. There are more digitations of the perivascular space of the capillaries of the primary plexus in the parenchyma of the posterior median eminence than noted in the anterior median eminence. In the posterior median eminence more axon endings are in contact with the perivascular space and axons containing granules of 1000 Å make synaptoid contacts with ependymal processes in the hypendymal and palisade layers.

The cell stations for fuchsinophilic (AF-positive) neurosecretory system are the cells of the nucleus supraopticus and nucleus paraventricularis. Arginine vasotocin and oxytocin are produced in them. There are fibre paths for those cells and axon terminals in two neurohaemal regions: the anterior median eminence and the pars nervosa. In both the neurohaemal areas arginine vasotocin and oxytocin are found. Release of the substances takes place from pars nervosa into the systemic blood and from the median eminence into the portal vessels. Release of these hormones is controlled by monoaminergic fibres as many such fibres as demonstrated by monoamine oxidase histochemistry and fluorescence microscopy come in contact with the perikarya of the AF + neurosecretory cells. Antidiuretic and vasopressor activity has been noted in arginine vasotocin. Oxytocin is diuretic and vasodepressor. Potency of arginine vasotocin for contractile action on the fowl oviduct is more in arginine vasotocin than in oxytocin. In some species of birds oxytocin leads to increase of fatty acids and blood sugar. The AF + system produces some adeno-hypophysiotrophic neurohormones which enter the pars distalis from the anterior median eminence through the anterior portal vessels.

The AF negative neurosecretory system comprises of cells whose localization is not definitely known but they have got axons and axon-endings in the



median eminence. This system produces releasing factors which reach the pars distalis and pars tuberalis through the adeno-hypophyseal portal system.

Ependymal cells of the median eminence secrete some substances into the third ventricle and absorb some material from the third ventricle. The processes of the ependymal cells extend to the capillaries of the primary plexus. "The presence of ventriculo-hypophyseal system is suggested in relation to hypothalamic control of the adeno-hypophysis."

Monoaminergic and non-monoaminergic cells form the nucleus infundibularis. The tubero-infundibular component of the releasing factor system plays an important role in the secretion of gonadotrophin. Both the neurons of the nucleus infundibularis send their axons to the palisade layer of the median eminence. The fibres form the tubero-infundibular tract. Monoaminergic neurons may produce gonadotrophin-releasing factors. At present there are evidences for gonadotrophin-releasing factors, a prolactin-releasing factor, an adrenocorticotrophin-releasing factor, and growth hormone-releasing factor in the avian hypothalamus. The chemical nature of the releasing factors and their location of formation in neural components are not known.

Calas(1975) studied the avian median eminence as a model for diversified neuroendocrine routes. In the duck median eminence, three main neuroendocrine routes may be described. LH-RH fibres in the hypothalamo-adeno-hypophyseal tract could be traced by immunocytochemistry in the external zone of the rostral and caudal median eminence (and also of the organum vasculosum laminae terminalis). Ultrastructurally there are two types of granulations, 1000 Å and 1200-1400 Å. Vasotocin-immunoreactive axons were recognized by the author in the hypothalamo-posthypophyseal tract. They are localized in the inner median eminence and also in the outer zone of the rostral ME. So, possibly this peptide has an action on the adeno-hypophysis. The labelling of the tanycytes observed 90 min after intraventricular injection of  $^3\text{H}$ -TRH is particularly intense after administration of  $^3\text{H}$ -histidine but weak after  $^3\text{H}$ -proline, suggesting a selective function of these cells in the transport and possibly the synthesis of this peptide.

"These three neuroendocrine routes might be linked by the monoaminergic innervation of the ME. Its noradrenergic component ends upon the dendrites and perikarya of infundibular neurons in the inner ME while its serotonergic component might affect in the outer ME the neurosecretory terminals and/or the feet of tanycytes. In fact, these two monoaminergic innervations seemed to exert an antagonistic control on a major neuro-endocrine regulation, the photo-gonado-regulation."

Ishii, Wada and Oota(1975) classified the neurosecretory axons of the median eminence and pars nervosa and identified the mediators contained in the granules of each class of axon group. The axons of median eminence of the horse were divided into five groups: A1, A2, B1, B2, and C having median



diameter of the granules of 93, 112, 130, 147 and 168 nm respectively. Group C axons could be further subdivided into C1 (164 nm granules), C2 (179 nm), and C3 (193 nm). Larger granule containing axons belonged to the fibre layer axons having terminations in the pars nervosa. In the equine *pars nervosa* the neurosecretory axons could be divided into B1 (123 nm granules), B2 (145 nm), B3 (158 nm), C1 (170 nm), C2 (180 nm), and C3 (190 nm). The number of group A1 axons in *pars nervosa* was very meagre. There was no group A2 axon in the *pars nervosa* of the horse but it was the most frequent axon group in the equine median eminence.

In the rat the authors found six axon groups (B1, B2, B3, C1, C2, C3) in the *pars nervosa*. The median granule diameters were strikingly similar to those noted in equine median eminence and *pars nervosa*.

In the bird Japanese quail (*Coturnix coturnix japonica*) the median granule diameters in the axons of the median eminence had a similar pattern as noted in equine median eminence. In this location there were six groups of neurosecretory axons. They are A1, A2, B1, B2, B3, and C1. The median granule diameters of these axons were slightly less than those in the horse. The authors examined only the external zone of the palisade layer of the median eminence with exclusion of the axons of the fibre layer. So they did not observe C2 and C3 axons in the median eminence of the Japanese quail. In the *pars nervosa* there were B1, B2, B3, C1, C2 and C3 axons and a small number of group A1 axons. There was no group A2 axon in the *pars nervosa* of the Japanese quail.

They concluded that the presence of group A2 axons is characteristic of the median eminence. This group is absent in the *pars nervosa*. C2 and C3 axons are present only in the *pars nervosa* and are not found in the external zone of the median eminence. In both these places B1, B2 and B3 axons have been noted (median eminence and *pars nervosa*). Group A1 axons are frequent in the median eminence but scarcely found in the *pars nervosa*. C1 axons are rare in the median eminence but not so rare in the *pars nervosa*.

Group A1 axons are noradrenergic. Granules of group A2 axons contain LRH. Other releasing or inhibiting hormones may also be contained in A2 axons. B1, B2, and B3 axons contain corticotrophin-releasing hormone (CRH) and neurohypophysial hormone (vasopressin in the mammal and vasotocin in the bird). The anterior median eminence of the Japanese quail contained more B2 axons than the posterior median eminence. Granules of group B2 axons contain vasotocin or vasopressin. Group C axons are thought to contain oxytocin in the mammal or mesotocin in the bird. MSH-inhibiting or releasing hormone may be contained in group C axons.

Regarding the regional differentiation of the median eminence, the authors proposed the following hypothesis in the bird. A single type of neurosecretory axons producing and releasing a single type of neurosecretory hormone, i.e. FSH-LH-releasing hormone may terminate in both the anterior and posterior median eminences. The hypothalamic FSH centre may send these axons in the



anterior median eminence or these may be regulated by neurons originating in the hypothalamic FSH centre. Hypothalamic LH centre may similarly send axons in the posterior median eminence. Following excitation of the FSH centre release of FSH-LH-releasing hormone occurs from the anterior median eminence and not from the posterior median eminence. As a result, there will be release of FSH from FSH cells which are thought to be situated only in the cephalic lobe. Similarly, when the hypothalamic LH centre is excited, release of LH from LH cells is noted which are believed to be distributed only in the caudal lobe. Thus the authors think that, "only a single or few kind of releasing hormone can regulate the release of multiple or a number of adenohypophysial hormones independently. Therefore, no or only slight difference in the axon population may be encountered between the anterior and posterior median eminence of the bird".

Controlling activity of the adenohypophysial cells by the specialized ependymal cells of the median eminence may take place either by a change in the concentration of a certain type of releasing hormone or active substance in the cerebrospinal fluid, or by a change in the transport activity of the ependymal cells of a certain area of the median eminence. For the latter type of control, the ependymal cells should be neurally controlled as well.

They concluded, "In general, the ordinary nervous system produces a limited kind of transmitter substance, although it controls a number of target organs. This system discriminates the information by developing the regional differentiation of the centre and the pathway conducting the information. On the other hand, the endocrine system uses a common pathway, the circulatory system and discriminates the information by the chemical diversity of the mediator. Thus, neurotransmitter substances are not so numerous in kind and not specific to the organ, but rather the hormone. The neurosecretory hormone of the median eminence seems to have an intermediate character between neurotransmitter substance and hormone in this sense".

Mikami(1975) made a correlative ultrastructural analysis of the ependymal cells of the third ventricle of Japanese quail (*Coturnix coturnix japonica*). Distinct regional variations in the ependymal cells of the wall and floor of the third ventricle of Japanese quail were noted by the author. The cilia of the dorsal two thirds of the ventricular wall have a peculiar club-shape, with increase in diameter towards the tip and terminate in a bulbous enlargement having a prickly surface appearance.

Two types of ependymal cells line the ventral wall and floor of the third ventricle: (1) nonciliated ependymal cells with large bleb-like protrusions, the apices of which are frequently of a pot-like form with a large opening or many fenestrations, and (2) tanocytes with a long basal process terminate on the subependymal capillaries or outer surface of the median eminence.

When ferritin was injected into the third ventricle, the bleb-like protrusion did not show an uptake, suggesting that secretion of some substance into the



third ventricle may take place. Uptake of ferritin could however, be demonstrated by the tanycytes in the ventrolateral wall and floor of the third ventricle. This suggests that tanycytes form a link between the CSF and portal circulation.

The authors further stated that the paraventricular organ appears as a distinct groove-like ependymal formation which is situated on the lateral wall of the lower third of the ventricle III. The organ consists of two types of non-ciliated ependymal cells and two types of nerve cells. A bulb-shaped ventricular process protrudes from the nerve cell situated in the subependymal layer and the process absorbs ferritin from the ventricle. The paraventricular organ may be a chemoreceptor and a secretory organ.

Nakai and Naito(1975) concluded that the ependymal cells in the frog median eminence have intracellular bidirectional (ascending and descending) transport activities of substances coming from both the hypophysial portal blood and the cerebrospinal fluid.

Kobayashi(1975) said that there is synaptic contact between the axons and the ependymal cells and such contacts have been noted in the median eminence among vertebrate species. Presumed monoaminergic axons possibly control the absorption of the ependymal cells in the median eminence. They found that in the hagfish and conger eel the ependymal cells of a region corresponding to the median eminence of higher vertebrate, absorb peroxidase when injected into the third ventricle.

They injected luteinizing hormone-releasing hormone(LRH) into the third ventricle of rats. There was an increase in serum LH. LRH from CSF was transferred from the third ventricle to the adenohypophysis. In demonstrating the route of transfer of peroxidase from the third ventricle to the adenohypophysis, Kobayashi injected  $^{125}\text{I}$ -LRH into the third ventricle of the rat. Autoradiographs demonstrated  $^{125}\text{I}$ -LRH in the ependymal perikarya and their processes, portal capillaries and adenohypophysis. It is possible that the ependymal cells of the median eminence can absorb gonadotrophin-releasing hormone from the CSF of the third ventricle. Active gonadotrophs have been observed in the adenohypophysial implants in the third ventricle of hypophysectomized rat(Szentagothai *et al.*, 1968) and in such implants in the fish (*Oryzias latipes*) (Kasuga, 1975- from Kobayashi, 1975).

Kobayashi(1975) prepared hypothalamic islands in Japanese quail by Halasz's knife. Great increase in the absorption of intraventricularly injected peroxidase was observed in such animals with division of axons entering into the median eminence. He concluded that the *ependymal absorption is under inhibitory control by some axons*. These axons are possibly of monoaminergic in nature and the perikarya are located outside the island.



*Cell types in the avian adenohypophysis*

These have been reviewed by Wingstrand(1951, 1963, 1966), Tixier-Vidal (1963), Tixier-Vidal and Follett (1973), and Wada(1975) and others.

The pars distalis can be divided histologically into a cephalic and a caudal lobe. The cells are arranged in cords or in acini and they are limited by a basement membrane. Majority of glandular cells contact the pericapillary space. Chromophobic cells are situated in the centre of cell groups and they have no direct relationship with the capillaries. They appear under the light microscope as conglomeration of nuclei(called *kernhaufen* of German authors). Two types of acidophilic cells are distributed in the two lobes of the pars distalis (Rahn and Painter,1941). The large A1 cells are situated in the caudal lobe and small A2 cells with fine granules are found in the cephalic lobe. In the cephalic lobe the cells are situated in groups or in longitudinal cords. In the caudal lobe plenty of pseudo-acini are seen. There are chromophobic and PAS+colloid droplets in the centre of the acini in the adult. Their increased number looks like a thyroid gland. Wingstrand(1951) discussed their significance. Tixier-Vidal *et al.*(1966) thought that the colloid represents a fibrous material which arises from the cytoplasm of the apical zones of gland cells. Wingstrand(1951) found some chromophobic cells of the pars distalis to contain a few argyrophilic granules after Bodian impregnation.

Tixier-Vidal and Follett(1973) distinguished two general classes of cells: cells with granules containing glycoproteins, and cells with granules containing simple proteins. Of the seven cell types in the bird pituitary, five belong to the above classes. Two other cell types form a third class having mixed affinities.

- (1) Proteinaceous cells (acidophils, serous cells). In this group there are alpha cells(caudal acidophils) and cephalic acidophils(erythrosinophilic cells).
- (2) Glycoprotein-containing cells. In this group there are beta cells (cephalic PAS+cells), delta cells(alcian blue+cells), and gamma cells (glycoprotein containing acidophils).
- (3) Mixed cell types consist of E cells and K cells (lead haematoxylin cells).

Wada(1975) studied the cell types in the adenohypophysis of the Japanese quail and effects of injection of luteinizing-hormone-releasing hormone.

Based on tinctorial properties the cell types were differentiated by Tixier-Vidal(1963), Gourdj(1965), Matsuo *et al.*(1968) and Tixier-Vidal *et al.*(1968).



Ultrastructural analysis was made :

<i>in</i>	<i>by</i>
domestic fowl	Mikami,1958 ; Payne,1965.
domestic mallard	Tixier-Vidal,1965.
the pigeon	Tixier-Vidal and Assenmacher,1966.
White crowned sparrow	Mikami <i>et al.</i> 1969, 1973.
Japanese quail	Tixier-Vidal <i>et al.</i> 1972.

Six cell types were identified by Wada(1975) in the adenohypophysis of the Japanese quail by examining alternate thick and thin sections by light and electron microscopy. The following findings are from Wada(1975).

#### *Cephalic gonadotrophic(GTH) cells*

These are PAS + basophils and can also be stained with alcian blue with PAS-AB-OG stain. They look red violet. With Herlant's tetrachrome the cytoplasm is blue with fine granules. Ultrastructurally the granules of these cells have many spherical, uniformly electron-dense granules of 150 to 300 nm (and sometimes upto 500 nm) in diameter. RER is scattered throughout the cytoplasm and in active cells these are dilated and they look deep violet in thick sections. In short-day birds these cells are round and chromophobic having some PAS + reaction. Ultrastructurally these round cells have smooth surface and the granules are small in number.

#### *Thyrotrophic(TSH) cells*

These basophilic cells in the cephalic lobe are pale blue in colour after PAS-AB-OG and are blue with Herlant's tetrachrome method. Ultrastructurally these cells have polymorphic, electron-dense granules of 150 to 250 nm in diameter. The rough endoplasmic reticulum(RER) is more or less dilated. No difference between the nonphotostimulated and photostimulated cells exists.

Destruction of the basomedial hypothalamus of chicken damaged a possible production site for TRH or the pathway of transport (Robinson *et al.*,1977).

Changes in serum triiodothyronine levels in the embryonic and post-hatching chicken, with particular reference to feeding-induced changes were noted by King *et al.*(1977). An increase in  $T_3$  levels occurred on the day of hatching (330 ng%) compared to those observed in seventeen and nineteen-day embryos. In one-day-old chick the levels were less than one-half the values observed closer to hatching. At 3 days post-hatching and 48 hours after food intake serum  $T_3$  levels increased 2.5 times compared to that in day-old chicks.  $T_3$  concentrations



decreased by 10 days of age (225 ng%). This value was more or less similar as observed on 33-44 days of age. Rise in serum  $T_3$  occurred with goitrogen treatments or changes in iodine intake. Serum  $T_3$  levels in PTU treated or methimazole treated 7-day-old cockerels were 60% of control values. The levels after PTU treatment for 25-36 days were only 10% of controls.

The findings of Thommes *et al.* (1977) demonstrate that in the developing chick embryo the adenohypophyseal-thyroid axis (plasma thyroxine) is functional on or about day 11.5 of incubation.

Klandorf *et al.* (1978) induced thyroxine and triiodothyronine release by TRH in the hen. Klandorf, Sharp and Duncan (1978) concluded that  $T_3$  is the metabolically active thyroid hormone in the chicken and  $T_4$  secretion is stimulated by a mechanism which depends on the dark period.

#### *Caudal gonadotrophic (GTH) cells*

This single type of basophil cell is alcian blue + and weak PAS +. It appears pale blue in colour with PAS-AB-OG. With Herlant's tetrachrome method the fine granules are blue in colour. When stimulated, these cells become tall with eccentric nuclei. Granules are of variable electron density and are 200-300nm (sometimes upto 400nm) in diameter. The well developed endoplasmic reticulum has many attached ribosomes. In short day birds, there is weak stainability of the cells and the cell is small. Granules are less electron-dense compared to those of photostimulated birds.

#### *Somatotrophic (STH) Cells*

These cells are situated in the caudal lobe. This is rather chromophobic with PAS-AB-OG and Herlant's tetrachrome method. They are round and large. Electron-dense granules are of 100-150nm in diameter. The granules are peripherally situated. The RER is vesicular. There are large electron-dense bodies which may be lysosomes. In nonphotostimulated birds the granules are large and more in number.

Hansen and Hansen (1977) used light and electron microscope to identify the pituitary cells containing growth hormone and prolactin in the pigeon (*Columba livia*). They employed immunoglobulin-enzyme bridge technique. Rabbit antisera were used against bovine growth hormone (anti b-STH) and ovine prolactin (anti-oPRL). The cephalic lobule contains  $A_2$  cells of Wingstrand. These cells are the  $\epsilon$  cells of Tixier-Vidal and Assenmacher. With Brookes' trichrome stain they were rose coloured. The  $A_1$  cells of Wingstrand or alpha cells of Tixier-Vidal and Assenmacher are situated in the caudal lobule. They are orange stained with Brookes' trichrome stain. With anti-bSTH reaction-positive cells are the  $A_1$  cells of the caudal lobule. They are oval or columnar having large, coarse granules. The nucleus is round or ovoid. Secretion granules in these cells are oval or rounded (200-300nm) ultrastructurally. With anti-oPRL



A<sub>2</sub> cells of the cephalic lobule are reaction-positive cells. They are oval, columnar or polyhedral containing fine granules. The nucleus is large, oval or irregular. Ultrastructurally the secretion granules are polymorphic (38-160nm when round; 80 × 500nm when rod-shaped).

Hoshino and Yamamoto(1977) studied the synthesis and release of growth hormone, prolactin and other proteins from the anterior pituitary of normal and dwarf chickens. Higher PRL synthesis and release has been noted in dwarf chickens. Caudal cells inhibit cephalic secretion. Slow-moving protein (SP) may be responsible for this inhibition and it plays a role in the development of dwarfism. SP may be a big GH or a type of carrier protein for some pituitary hormones.

Camper and Burke(1977) concluded that LH or FSH has a steroidogenic action on the turkey ovary eliciting rapid increases in serum progesterone and estradiol.

Seasonal variations in the circulating concentrations of growth hormone in male Peking duck (*Anas platyrhynchos*) and teal (*Anas crecca*) and correlations with thyroidal function were observed by Scanes *et al.*(1980).

#### *Prolactin(PRL) cells*

These acidophilic cells are located in the cephalic lobe. With Herlant's tetrachrome method they are faintly pink. The granules are large (300 to 500nm). There is well developed endoplasmic reticulum.

Nicoll(1974) classified the actions of prolactin related to reproduction in birds. They are: production of crop milk, formation of brood patch, antigonadal, premigratory restlessness, parental behaviour, synergism with steroids on female reproductive tract, and suppression of sexual phase of reproductive cycle. Growth promotion actions on specific target cells or tissues are: proliferation of pigeon crop-sac mucosa, epidermal hyperplasia in brood patch, feather growth, and development of female reproductive tract. Prolactin (with corticosteroids) stimulates nasal(orbital) salt gland secretion. During formation of brood patch prolactin has synergism with ovarian or testicular steroids. In parental behaviour prolactin has possible progesterone synergism. It has synergism with estrogens and progestins on female reproductive tract. With sex steroids (?) prolactin is antigonadotrophic.

Prolactin and LH levels were studied by Burke and Dennison(1980) in female turkeys (*Meleagris gallopavo*) during a photoinduced reproductive cycle and broodiness.

An intense crop-sac response in the pigeons, was observed by Nistico *et al.*(1980) after 3 or 5-day systemic treatment with reserpine, haloperidol, and (±) sulpiride. A much lower dose of haloperidol, clozapine, and the isomers of sulpiride given intraventricularly(III) for three consecutive days produced a marked crop-sac response and stimulation of pituitary lactotrophs.



*Corticotrophic (ACTH) cells*

These cells are situated in the cephalic lobe. They are chromophobic to PAS-AB-OG and Herlant's tetrachrome. The few electron-dense granules are 100-150nm in diameter. Large vesicles are formed by endoplasmic reticulum. In nonphotostimulated birds the granules are more in number than those in the photostimulated birds.

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Gonadotrophic cells in both lobes were strongly activated after exposure to long daily photoperiods. The cells became larger, loaded with granules. ACTH cells became vacuolated with sparse granules. After synthetic LRH injection (10 mcg/0.2ml per day) for 10 days to the nonphotostimulated quail, stimulation of certain numbers of gonadotrophic cells in both the lobes, was noted though the response of the cells was less than that induced by photostimulation. No change was observed in other cell types.

Wada(1975) further discussed that the cephalic gonadotrophic cells correspond to the beta cells of Tixier-Vidal *et al.*(1968, 1972) and of the Pekin duck (Tixier-Vidal and Assenmacher, 1966). They also correspond to the Type A gonadotrophic cells of Mikami *et al.*(1969) and GTH cell of Mikami *et al.*(1973) in the white-crowned sparrow. These cells respond to LRH injection. Therefore, they are thought to be gonadotrophic cells. They produce FSH because in this cephalic lobe Brasch and Betz(1971) found maximum FSH activity in the chick.

TSH cells are similar to the delta cell of Tixier-Vidal *et al.*(1968). Brasch and Betz(1971) found TSH activity only in the cephalic lobe of chicken. So these cells may be TSH cells.

Caudal gonadotrophic cells were thought to be TSH cells by Tixier-Vidal *et al.*(1968). But ultrastructurally these are LH cells as they respond to LRH injection and LH activity in the chick has been demonstrated to be mostly present in the caudal lobe(Brasch and Betz,1971).

STH cells are same as alpha cells of Tixier-Vidal *et al.*(1968). These alpha cells are same as somatotrophic cells of Mikami *et al.*(1969, 1973) except the difference in the size of the granules.

The prolactin cells of the cephalic lobe are erythrosinophilic and same as eta cells of Tixier-Vidal *et al.*(1968).

ACTH cells of Wada are epsilon cells of Tixier-Vidal *et al.*(1968). Ultrastructurally these cells correspond to the ACTH cells of the duck after metopirone treatment (Tixier-Vidal,1965) and ACTH cells of White-crowned sparrow after adrenalectomy(Mikami *et al.*,1969).



Kalliecharan and Hall(1977) found that several pathways of biosynthesis of corticosteroids are in action in the adrenal gland of the embryonic chick. ACTH added to the incubation medium stimulated steroid secretion into the medium but it did not affect the levels within the glands.

Anterior infundibular nuclear complex controls FSH secretion and the posterior complex controls LH secretion.

Though there is no intermediate lobe in the avian pituitary, there are MSH-secreting cells(K cells). These cells are situated mainly in the cephalic lobe and are intensely lead haematoxylin positive. With Herlant's tetrachrome these cells are dark blue and so they may be considered as basophils. The phospholipid and tryptophan content is high in these cells in the Japanese quail. Their glycoprotein content is low. These cells contain round, dense granules(400-500nm). The endoplasmic reticulum is well developed and there are flattened sacs lined by ribosomes(Tixier-Vidal and Follett,1973).

Chromatographic and electrophoretic characterization of melanocyte-stimulating substances in the duck pituitary was made by Estivariz *et al.*(1980). They concluded that the bulk of melanotrophic activity of the duck pituitary corresponds to a peptide which behaves as  $\alpha$ -MSH in the analytical systems used. In human beings and in the fin whale pars intermedia is not present and  $\alpha$ -MSH is absent also, but good amount of  $\alpha$ -MSH is present in the duck pituitary lacking a pars intermedia.

Iturriza *et al.*(1980) obtained findings to suggest coexistence of  $\alpha$ -melanocyte-stimulating hormone and adrenocorticotrophin in all cells containing either of the two hormones in the duck pituitary. They also speculated that in birds *intermedia-like cells* replace the pure corticotrophs which should be present in the pars distalis. It is also possible that ACTH is a prohormone for  $\alpha$ -MSH.

Wada and Asai(1976) studied immunohistochemical localization of LH-producing cells in the adenohypophysis of the Japanese quail (*Coturnix coturnix japonica*). The LH cells were identified immunohistochemically using anti-chicken LH serum and horseradish peroxidase-labeled goat anti-rabbit gamma globulin serum. The LH cells are situated in the caudal lobe of the pars distalis. They are elongated and are situated towards the sinusoids, when they are active. The changes in the size of LH cells are directly related to changes in circulating LH levels after castration or photostimulation. These immunocytochemically identified LH cells were only stained by alcian blue with PAS, alcian blue and orange G.

PAS positive gonadotrophic cells of the cephalic lobe had slight staining immunohistochemically, if at all, using anti-chicken LH serum and so they may be FSH producing cells. Another alcian blue + basophilic cell of the cephalic lobe is also stained immunocytochemically with anti-chicken LH serum. They



may be TSH cells as the anti-chicken LH serum cross reacted with LH and TSH but only slightly with FSH. The possibility that these are TSH cells is also from the light and electron microscopic observations.

By use of anti-HCG anti-serum (Ravona *et al.* 1973) or a chicken anti-LH-anti-serum (Sharp, 1974) it has been confirmed that the distribution of the gonadotrophic cells is throughout the pars distalis.

Tsai and Chadwick (1977) described the cytology of the pituitary gland of the fowl *Gallus domesticus*. Pituitary cells containing growth hormone and prolactin were identified by Hansen (1977) with the help of light and electron microscope using the immunoglobulin enzyme bridge technique.

Harvey *et al.* (1978) studied the effect of thyrotrophin-releasing hormone (TRH) and somatostatin (GHRH) on growth hormone and prolactin secretion *in vitro* and *in vivo* in the domestic fowl *Gallus domesticus*. The same authors in 1979 observed the influence of sex and breed on growth hormone and prolactin secretion in the domestic fowl. Regarding the action of prolactin on the uropygial gland of chicks Chakraborty *et al.* (1979) thought that although prolactin can promote mitosis in the uropygial gland of chicks, it does not appear to stimulate the glandular function.

Burke *et al.* (1979) prepared and studied the properties of LH subunits from the turkey (*Meleagris gallopavo*) and their recombination with subunits of ovine LH. Turkey LH is made up of two dissimilar subunits. "Each of the subunits has substantial chemical homology with those from ovine LH. The species specificity of these two tetrapod luteinizing hormones appears to be associated with the  $\beta$ -subunit". The same authors in 1979 isolated and characterized LH and FSH from pituitary glands of the turkey. In radioreceptor assays turkey and chicken FSH are similar. Turkey LH was consistently more potent than either avian FSH in competing for FSH-binding sites. Comparatively chicken LH had relatively low activity. The authors suggested, "the evolution of the structure of active sites in turkey LH has involved convergence on those of the FSH molecule".

From a comparative study of the annual cycles in sexual and thyroid function in male Peking ducks (*Anas platyrhynchos*) and teal (*Anas crecca*) Jallageas *et al.* (1978) came to the conclusion that "in both the species a biphasic sexual cycle is found with (1) a main reproductive phase in spring, associated with sexual displays in the teal, (2) a decrease in all sexual parameters from June through July, and (3) a transitory sexual recovery in August-September. Plasma thyroxine cycle showed annual maximum in June-July. An additional annual peak of plasma thyroxine was found in the teal in December".

Assenmacher and Tixier-Vidal (1965) studied the hypothalamic-pituitary relations in the duck which included ultrastructural findings of the FSH and LH cells and prolactin cells. An increase in illumination, either seasonal or artificial stimulates the activity of the FSH, LH and prolactin cells.





Electron microscopic observations of gonadotrophs and prolactin cells under different experimental conditions in the male duck have been made by them.

In birds an absolute stimulatory control is exerted by the hypothalamus over the FSH and LH cells. There is a stimulatory control of the prolactin cells by the hypothalamus. They however, could not detect any proof of hypothalamic inhibition of prolactin cell activity. Certain degree of autonomy of the prolactin cells could be detected and activity remained even after disruption of neuro-vascular connections with the hypothalamus.

Wada(1979) obtained results to suggest "that LH release is induced when light impinges on the circadian photosensitive phase which is set by external lighting schedules". Balthazart *et al.*(1979) suggested, "the metabolic changes at the pituitary level may play some role in the induction of the increased responsiveness to LHRH which can be observed in quails after exposure to 7 long days".

Production and secretion of sex steroid hormones by the testes, the ovary, and the adrenal glands of embryonic and young chicken (*Gallus domesticus*) was studied by Tanabe *et al.*(1979). Plasma LH concentrations were high in male and female embryos. They dropped to a low level at hatching and again increased subsequently. Adrenal glands play an important role for production and secretion of testosterone compared to the testes or ovary in the embryonic chicks. Embryonic testes are less active than the embryonic ovary for testosterone and estradiol production. The testis and ovary produce and secrete more sex steroids than the adrenal gland after hatching.

As in mammals androgens play an important role in avian (Japanese quail) spermatogenesis(Brown and Follett,1977). Probably FSH is also required for full spermatogenesis and testicular growth. Testosterone exerts a differential effect on the quail testis(Desjardins and Turek,1977). "It depends, in part, upon the amount of androgen administered".

Bicknell and Follett(1977) noted luteinizing hormone releasing activity in the quail hypothalamus during photostimulated sexual development. Increase in LH secretion after transfer to photostimulatory day lengths was not associated with any major changes in LHRH of basal hypothalamic tissue. Castration did not change LHRH activity during one 5-week experiment. Castrated birds maintained on long days for one year showed the highest level of releasing activity found in any quail.

In sera from immature cockerels diurnal variations in LH and prolactin were demonstrated by Scanes *et al.*(1980). These patterns of hormone secretion were significantly altered by pinealectomy. Diurnal variations in growth hormone concentration was not seen in intact or pinealectomized birds.



Jenkins *et al.* (1978) studied the effects of vertebrate gonadotrophins on androgen release *in vitro* from testicular cells of Japanese quail and a comparison with their radioimmunoassay activities was made. They concluded that LH primarily acts on the testicular cells of quail. FSH is nonsignificant in acute release of androgen from the cellular component of the testis. The results also confirm the general specificity of the avian LH and FSH radioimmunoassays.

From *in vivo* results Maung and Follett (1978) came to the conclusion that in the quail, pituitary LH controls peripheral androgens and FSH has no significant role in the acute release of testosterone from the mature testis.

FSH and testosterone induce FSH receptors in the testis of immature Japanese quail and thereby increases the sensitivity of the testis to FSH. This is a type of self-potentiating action of FSH. This mechanism along with synergism between FSH and testosterone most likely helps the extremely rapid increase in testicular weight in photoperiodically stimulated birds (Tsutsui and Ishii, 1978).

An inverse relationship between gonadal activity and  $T_3$  activity exists (Oishi and Konishi, 1978).

Difference of Japanese quail LHRF from mammalian LHRF was revealed by biological and immunochemical studies (Hattori *et al.*, 1980). The observations indicated a species-specificity among these LHRFs. Presence of immunoreactive substance(s) in the quail hypothalamus was detected by immunohistochemical method using antisynthetic LHRH serum and peroxidase-labeled anti-rabbit  $\gamma$ -globulin serum.

Diurnal variation in plasma LH levels in the domestic fowl (*Gallus domesticus*) was observed by Scanes *et al.* (1978). Increase in LH concentration occurred shortly after the starting of the dark period in both sexes.

Harvey *et al.* (1978) studied the effect of thyrotrophin-releasing hormone (TRH) and somatostatin (GHRH) on growth hormone (GH) and prolactin (Prl) secretion *in vitro* and *in vivo* in the domestic fowl (*Gallus domesticus*). Intravenous administration of TRH in both the conscious and anaesthetized immature chicken significantly increased plasma GH. Somatostatin when simultaneously administered to conscious birds led to significant reduction in the magnitude of GH response to TRH treatment but no effect was observed on the basal levels of plasma GH. High level of plasma GH could not be maintained by repeated TRH injection. In none of these experiments Prl secretion was stimulated. In anaesthetized birds plasma Prl level was found to be depressed by TRH treatment. The effects of TRH and somatostatin on GH secretion by an *in vitro* dispersed pituitary cell suspension system were very similar to the *in vivo* studies, Prl release *in vitro* was stimulated by TRH, in contrast to the *in vivo* studies, and the response was dose related. *In vitro* Prl release (basal) was not influenced by somatostatin, but it significantly inhibited the response to TRH treatment.



Increase in plasma and pituitary levels of LH and FSH was found by Davies and Follett(1980) when quail was transferred to long days. For mediation of this photoperiodic response, preoptic region(POR) and posterodorsal part of the infundibular nuclear complex(PD-INC) are essential. In nonphotostimulated immature birds low levels of LHRH release can be regulated autonomously by INC. The authors combined the techniques of electrical stimulation and electrolytic lesioning and demonstrated that POR stimulation leads to an increase in LHRH secretion in quail where the photoinduced release was blocked by PD-INC lesion. Similarly stimulation of preoptic region in immature birds stimulates secretion of LHRH. "These results suggest a direct link, perhaps neurosecretory, between the preoptic region and the median eminence".

Doi *et al.*(1980) suggested that the onset of light may be a signal for the increase of plasma LH and progesterone and follicular progesterone which are responsible for the induction of ovulation in the quail (*Coturnix coturnix japonica*).

Testosterone treatment blocks the termination of the gonadal photorefractory condition in white-throated sparrows maintained on short days(Turek *et al.*,1980).

Ionic and endocrine factors influence the secretion of LH by chicken anterior pituitary cells *in vitro*(Luck and Scanes, 1980).

The observations and data of Wingfield *et al.*(1980) suggest that the increase in plasma levels of LH and  $17\beta$ -hydroxy- $5\alpha$ -androstane-3-one in both sexes of white-crowned sparrow, *Zonotrichia leucophrys* may play a role in the development of song and social behaviour that permits the integration of the young into winter flocks.

Tanabe *et al.*(1980) suggested that the onset of darkness is closely linked to the induction of plasma LH and progesterone increases and lights off may be the signal for the induction of ovulation in the female duck (*Anas platyrhynchos domestica*).

El Halawani *et al.*(1980) observed that norepinephrine-containing neurons facilitate central mechanisms which release LH in response to neural inputs involved in the photoperiodic stimulus and that activation of dopamine-containing neurons is capable of inhibiting this release and inducing testicular regression of Japanese quail.

Photorefractoriness is not likely to be caused by some form of *exhaustion* of the hypothalamo-hypophysial unit in willow ptarmigan (*Lagopus lagopus lagopus*) (Stokkan and Sharp,1980). In these birds seasonal breeding results from an interaction between a direct effect of day length on LH secretion and day length-induced changes in the sensitivity of the hypothalamus to the inhibitory feedback action of adrenal and testicular steroids.





Tixier-Vidal and Assenmacher(1965) studied on some aspects of the pituitary-thyroid relationship in birds (adult male Pekin ducks). Thyrotrophic cells in electron-microscopic study showed abundant ergastoplasmic cisternae and very diffuse secretory granules. The thyrotrophs in ectopic grafts in the hypophysectomised duck became chromophobic and difficult to identify even by electronmicroscopy.

Castration greatly stimulates the thyrotrophs. They become hypertrophied, vacuolated and more degranulated than noted after throidectomy. The thyroidal hypertrophy is of thyrotrophic origin. Depressive effect of the male hormones is on the thyrotrophs which become *freed* in their absence.

Intact vascular hypothalamo-pituitary connections is needed for the stimulatory effect of castration on the thyrotrophs. The effect fails to occur if castration is preceded by section of the pituitary-portal vessels. It is also true for the depressive action of sexual activity on thyroid function. So inhibitory action of sex hormones acts at the hypothalamic level.

The stimulatory effect of light on the thyrotrophs is very striking. The cells look voluminous and turgescnt. Ultrastructurally the entire cytoplasm is invaded by swollen Golgi apparatus and by plenty rounded ergastoplasmic cisternae, evenly distributed. Granules are less and small and they occur mainly at the cell periphery. Synthesis and release of the hormone seems to follow a rapid rhythm.

Light stimulation of the thyrotrophs is abolished by the section of the pituitary-portal vessels. So the effect is probably mediated via the hypothalamus.

They concluded that permanent light and permanent darkness exert antagonistic effects on thyrotrophic activity. One is stimulatory and the other is inhibitory. Light acts through the hypothalamo-hypophysial pathway. The effect of darkness is similar to that of disruption of the hypothalamo-hypophysial pathway.



Table showing onset of hormonal production and secretion of hormones in embryonic chicken pituitary gland and blood

Authors	GH		PRL		ACTH		Days of incubation
	Pituitary	Blood	Pituitary	Blood	Pituitary	Blood	
Enemar (1967)	Biological assay method : caudal lobe						15 days
Ferrand <i>et al.</i> (1974)					Immunohistology		9 days
Fellman <i>et al.</i> (1975)					Immunopositive cells in the cephalic part (fluorescent antibody method). Anti-ACTH sera generated against 1-24 and 17-39 fragment of ACTH.		8 days



Table showing onset of hormonal production and secretion of hormones in embryonic chicken pituitary gland and blood—(Continued)

Authors	GH	Days of incubation	PRL	Days of incubation	ACTH	Days of incubation
	Pituitary		Pituitary		Pituitary	
	Blood		Blood		Blood	
Jozsa <i>et al.</i> (1979)	Few polygonal immunopositive cells in the caudal segment. GH antisera raised against the hormone and purified from chicken adenohypophyseal tissue.	12 days	PRL + cells (round or polygonal) in the Rathke's pouch. Cells are roughly granulated.	6 days	Immunopositive cells in the cephalic part. Anti-ACTH sera raised against 1-28 fragment of porcine ACTH	7 days
			PRL + cells equally distributed in cephalic and caudal lobes	15 days		
	GH immuno + cells completely filled the caudal segment and a few cells were located in the cephalic part.	45 days post-hatching	PRL antisera raised against the hormone and purified from chicken adenohypophyseal tissue. Anti-PRL serum cross-reacted with other trophic hormones except ACTH. Antibodies were raised against the common $\alpha$ -chain of LH and TSH. There is a possibility of cross-reaction with LH, TSH and FSH.			



Table showing onset of hormonal production and secretion of hormones in embryonic chicken pituitary gland and blood—(Concld.)

Authors	Pituitary	Blood	GH	Days of incubation	Pituitary	PRL	Days of incubation	ACTH	Blood	Days of incubation
Harvey, Davison, and Chadwick (1979)		Plasma GH was measured with specific homologous radioimmunoassay for chicken GH. Blood was collected from umbilical veins in embryos and by frontal cardiac puncture in neonates.	Detectable (6ng/ml) after 17 days. 16ng/ml on the day after hatching. 50ng/ml in 3rd day post-hatching.			Specific homologous radioimmunoassay for chicken prolactin.	Low plasma prolactin level (<6ng/ml) in 9, 11, and 13 days. 78ng/ml after 17 days. 37ng/ml in 19 days. 140ng/ml on the day after hatching. 49ng/ml in 5 day-old chicks.			



## CHAPTER 15

### COMPARATIVE ASPECTS OF THE EVOLUTION AND HOMOLOGY OF THE PITUITARY

The general morphological plan of the avian pituitary was described by Wingstrand(1951) as follows :

1. The caudal lobe is formed by the aboral lobe of Rathke's pouch, and it includes also the walls, which in the early stages look like an intermedia.
2. The cephalic lobe is formed by the oral lobe of Rathke's pouch and its unpaired processes.
3. The lobuli lateralis form the pars tuberalis. The basis of the lobi is situated in the furrow between the caudal and cephalic lobes and fuses with the pars distalis in this location. These basal parts of the lateral lobes can sometimes be recognized in the adult as a pars tuberalis interna. The lobi lateralis fuse more or less completely in the adult just dorsal to the pars distalis and form the portal zone. Their distal parts cover the surface of the median eminence and adjacent parts of the diencephalon, and a second fusion usually takes place behind the indundibular stem.

A comparison was made between the structural plan of the adenohypophysis in birds and reptiles by Wingstrand(1951)(fig. 15.1). It shows that the caudal lobe of the adult avian pituitary corresponds to the intermedia and the adjacent part of the pars distalis in reptiles because the material in both cases is supplied by the aboral lobe. The cephalic lobe in the avian pituitary corresponds to the rostral part of the reptilian pars distalis, and this part is well developed in reptiles. The lobi lateralis give rise to a pars tuberalis in birds, crocodiles, chelonians and Rhynchocephalia, but do not form a tuberalis in the snakes, in which they are indistinct already in small embryos, and they are partly or completely reduced also in lizards.

When the comparison was made between mammals(fig. 15.2) and birds, it was found by Wingstrand(1951) that the avian caudal lobe corresponds to the entire epithelial gland of mammals, with the exception of course of the pars tuberalis which has its equivalent in birds, and a very restricted portion which may correspond to the cephalic lobe. The latter which is derived from the oral lobe is represented by some vestigial part of tissue near the rostral end of the mammalian gland or, in other cases, under its rostroventral surface.



The metaadenohypophysis, like the pars intermedia of tetrapods is formed by the aboral lobe of Rathke's pouch in cyclostomes, elasmobranchs and among actinopterygians at least in the herring. It is situated in close contact with the neural lobe containing majority of neurosecretory fibres (distale neuroadenohypophysäre kontaktfläche of Spatz, Diepen and Gaupp, 1948). Nerve fibres from neural lobe invade the pars intermedia (metaadenohypophysis of fishes) in tetrapods, lungfishes, actinopterygians and elasmobranchs. The mesoadenohypophysis has been homologized with the pars distalis and the proadenohypophysis either with the pars tuberalis or has been considered to be a part peculiar to fish or the problem is as yet unsettled. Mesoadenohypophysis has been considered as the homologue of the pars tuberalis by de Beer (1926) and Diepen (1954, 1955, 1962). This part is in contact with the anterior, nonneurosecretory fibres of the neurohypophysis which is called an eminentia (Infundibulum). Consideration of pro or mesoadenohypophysis as pars tuberalis is unsatisfactory for two reasons (Wingstrand, 1966) : (1) In tetrapods the lateral lobes in embryos develop into the pars tuberalis. In fish and cyclostomes the pro and mesoadenohypophysis are unpaired, and (2) it is not always true that the anterior part of the adenohypophysis or the part having functional contact with the median eminence should always be the pars tuberalis (Enemar, 1960). In the tetrapod embryo the unpaired anterior process of the oral lobe or the oral lobe itself fits with these requirements better. Enemar showed that contact between the anterior process of the oral lobe and the brain occurred in reptiles and birds. Portal vascular connection is formed at the area of contact. The lateral lobes appear in this area after the vascular connection has already been established, or they do not develop at all. The anterior process is vestigial, or absent in mammals. At the anterior end of the gland and at the base of the pars tuberalis, the oral lobe is represented as a small rudiment. This originates in the anterior end of the gland in mammals, but not in other amniotes.

Herlant (1954) tentatively compared the pro and mesoadenohypophysis of fish-like vertebrates with the cephalic and caudal lobes of the pars distalis of reptiles and birds. Pars intermedia or metaadenohypophysis forms the posterior contact zone with the neurohypophysis. In all vertebrates this corresponds to the top of Rathke's pouch. The anterior end of the pituitary comes in contact with the brain wall further anteriorly and receives the portal vessels. It is the basal part of Rathke's pouch, the oral lobe. An anterior process develops from the oral lobe. It forms the anterior end of the gland. In mammals the anterior end of the gland contains the remnants of the oral lobe. The pars tuberalis is situated more anteriorly because the oral lobe is rudimentary. In reptiles and birds the anterior process of the oral lobe forms the anterior end of the gland. In some cases the anterior process may be compact. In snakes the pars tuberalis may be completely absent.

In amphibians the pars tuberalis develops from the lateral lobes on each side. It is either of U-type or of O-type of investigators from Japan. U-type is



found in urodela and O-type has been detected in anura. Wingstrand(1966) thought that the unpaired part between the lateral lobes develops as an unpaired process and most probably it represents the anterior process since it receives the portal vessels and is attached to the eminentia. In anura the pars tuberalis (O-type) on each side is largely independent of the portal circulation. In lungfishes no pars tuberalis develops and the remaining parts develop in the same way as in amphibians(figs. 15.3 & 15.4).

The anterior end of the adult gland of the elasmobranchs (figs. 15.5a & b) is formed by an anterior process (the *Vorraum* of Woerdeman) from Rathke's pouch. Portal vessels proceed from the eminentia to the anterior end of the gland. The ventral lobe is formed by a pair of lateral processes which seem to be the lateral lobes of amniotes and thus may be a true homologue of the pars tuberalis.

The pituitary of actinopterygians (figs. 15.6A, B, C) is oriented similar to that of sharks. The hollow epithelial stalk in Elops, Chanos and young Clupea joins the anterior end of the gland. In Polypterus the duct joins the proadenohypophysis. It indicates that this part is the proximal (oral) lobe of Rathke's pouch. The proadenohypophysis in Polypterus receives blood from the median eminence through the vascularized ligament situated in front of the gland. In Acipenser similar condition exists (Wingstrand,1966).

In Petromyzon(fig. 15.7) the epithelial stalk is continuous with the proadenohypophysis till the time of metamorphosis. It indicates that the proadenohypophysis is the oral part of the pouch. The remaining part of the gland is homologous with that of other vertebrates.

As the anterior end of the pituitary was shown by Wingstrand(1966) to be morphologically comparable in different vertebrates, he supported the interpretation of Herlant(1954) that the cephalic lobe of reptiles and birds is comparable to the proadenohypophysis. The identification of mesoadenohypophysis as the caudal lobe is difficult on embryological basis. Histologically the proadenohypophysis in teleosts, sharks and cyclostomes differs markedly from the cephalic lobe of reptiles and birds.

#### *Zona tuberalis*(fig. 15.8) :

This histological zone is present in mammals and it is a modified area near the entrance of the portal vessels (Wingstrand, 1956). It includes large parts of the gland which are derived from the embryonic aboral lobe(Wingstrand, 1951). Similar zones in reptiles only include a small part of the oral lobe. Therefore it appears advisable not to attribute too much morphological value to these comparisons of histological regions (Wingstrand,1966).



There is total or partial absence of acidophils from Dawson's zona tuberalis and it consists mainly of chromophobes and basophils.

The zona tuberalis of the *cat* is mainly made up of the aboral lobe. The remnants of the oral lobe and the very basal parts of the lateral lobes may also be included. In the *rabbit* the zona tuberalis consists of the modified area of the aboral lobe and of the oral lobe. The part played by the lateral lobes in the formation of the zona tuberalis depends on the choice of limits for the zona in this species (Wingstrand, 1951). The zona in the bird corresponds to the less stainable caudal lobe. Branches of the portal veins are primarily distributed within the zona tuberalis. Glandular cells in this part of the pituitary are influenced by the portal blood (Dawson, 1948). The distribution of portal vessels can hardly be used as a criterion for homology between the parts known as zona tuberalis in different mammals (Wingstrand, 1951). In adult birds the portal vessels pass into the cephalic and caudal lobes as soon as they reach the surface of the gland. Blood from the rostral median eminence passes into the cephalic lobe. The caudal lobe is supplied by blood from the caudal median eminence. The lateral lobes continue into the depth of the gland as a *pars tuberalis interna* on each side. The distribution of portal vessels in birds is not along the basal parts of the lateral lobes. Therefore it cannot be used as a criterion for the homology of the main part of the zona tuberalis (Wingstrand, 1951). "*The cephalic lobe in birds does not correspond to the main part of the zona tuberalis of mammals—viz. the part derived from the aboral lobe—but only to the part which represents the vestigial oral lobe. If the basal parts of the lateral lobes contribute to the zona tuberalis, as they probably do in the cat they are of course directly comparable to the pars tuberalis interna in birds*" (Wingstrand, 1951).

The delicate cell-strings along the rostro-ventral surface of the pars distalis of the *cat* are remnants of the ventral portions of the lateral lobes which have coalesced with the pars distalis. This is called *pars tuberalis interna* as opposed to the *pars tuberalis externa* which is the proper pars tuberalis (Hanström, 1966). The basophilic-chromophobic *zona tuberalis* of Dawson is commonly found in the *Eutheria* but Hanström saw it in only one specimen of the *Tachyglossus*. "The term *pars tuberalis interna* ought to be used for those portions of the pars distalis in adult mammals which prove to be remnants of the lateral lobes of the embryonic Rathke's pouch, while the term *zona tuberalis* should be used for a chiefly chromophobic part of the median rostro-ventral area of the pars distalis without any reference to its ontogenetical or comparative-anatomical significance. It is possible that future embryological investigations will show that in several instances the region which is at present called a zona tuberalis is actually a *pars tuberalis interna*" (Hanström, 1966).

Another characteristic of the pituitary of the *rabbit* is a small circumscribed area within the pars distalis at the rostral pole of the pars intermedia. This



area is rich in connective tissue and blood vessels. It is not present in great majority of other mammals (Hanstrom, 1966).

Acidophil zones are rich in acidophils. Zones poor in acidophils are called basophil zones. The cell population in the basophil zones may not be large proportion of basophil cells but in some species it may be chromophobes. As the zonal distributions are permanent, the cell types are stable and as they are once differentiated, re-differentiation into other types does not occur. Functional cell types can be identified by the assay of the hormone contents of different hypophysial zones in large mammals (Purves, 1966).

The posterior part of the post-optic hypothalamic floor is evaginated to form an unpaired process, the *saccus infundibuli*. In majority of the vertebrates this saccus becomes forked at the end, forming a pair of primary branches. The saccus infundibuli is related to the top of the notochord, the vena retro-hypophysis and the top of Rathke's pouch (Wingstrand, 1959). In *Petromyzon* a saccus infundibuli with a pair of primary branches is also present. The saccus infundibuli forms the neural lobe of the pituitary in amniotes. In elasmobranchs and primitive actinopterygians the neural lobe develops in the ventral wall of the saccus infundibuli and in the space between the primary branches. The saccus vasculosus develops from the dorsal parts. In amphibians and lungfish (except the *Gymnophiona*) similar conditions are found. The ventral wall of the saccus infundibuli and the wall between the primary branches develop into the neural lobe. The dorsal walls remain undifferentiated. The saccus infundibuli of teleosts mainly forms the saccus vasculosus. Morphologically the neural lobe of amniotes may be homologized with the saccus vasculosus of fish. Functionally the neural lobe of amniotes may be compared with the neurosecretory part of the neurohypophysis of fish. The anterior part of the neurohypophysis is connected with the pro and mesoadenohypophysis in the majority of aquatic vertebrates and the posterior part is in contact with the metaadenohypophysis. The posterior part is called the neural lobe also in the fish and the anterior part may be called the *eminentia mediana* as it contains less neurosecretory material. *Eminentia* is present in *Myxine*, elasmobranchs, *Polypterus*, teleosts. In teleosts the humoral link between the median eminence and the adenohypophysis is greatly shortened or may be substituted by direct synaptic connections (Wingstrand, 1966). The neurohypophysis can be divided into *eminentia* and neural lobe in the lungfish and tetrapods. In the hagfish, *Eptatretus burgeri* the fine structure of the ventral wall of the neurohypophysis has characteristics common to the median eminence of higher vertebrates. The dorsal wall has characteristics common to the *pars nervosa* of higher vertebrates. AF + material is present only in the dorsal wall. The portal vessels have developed poorly in this species (Kobayashi, 1972).



### Phylogeny

In most elasmobranchiomorphs and actinopterygians a typical saccus vasculosus with crown cells is present. It is absent in cyclostomes and tetrapod-lungfish. Wingstrand(1966) thought that the saccus vasculosus may have evolved in some common ancestor of actinopterygians and elasmobranchs.

In cyclostomes the adenohypophysis has an extra-stomodæal origin in association with the olfactory organ (figs. 15.7 & 15.9). The fossil cephalaspidomorphs have a dorsal opening from the olfactory organ like *Petromyzon* (Stensio,1958). In *Myxine* the nasohypophysial opening is at the anterior end of the head but outside the mouth. A similar preoral opening was present in the fossil *Pteraspidomorphs* (Stensio,1958, pp. 359, 360). In gnathostomes the adenohypophysis has a separate origin in the roof of the stomodeum.

All forms do not possess a hypophysial cavity. When present, it is in contact with the glands and separates the pars anterior from the pars intermedia. In *Petromyzon* there is a hypophysial cavity but it is separated from the glands by connective tissue and it does not separate the pars anterior from the pars intermedia. In *Petromyzon* (and other cyclostomes) and in *Polypterus* the hypophysial cavity opens to the exterior in the adult. This may be regarded as a persistence of Rathke's pouch (de Beer,1926). In cyclostomes the hypophysial cavity is very large. In *Petromyzon* the differentiated glands form the roof of the hypophysial cavity when it appears. In this form the hypophysis arises from in front and proceeds backwards. The hypophysial cavity is horizontal. The floor of the infundibulum is horizontal also. In higher vertebrates the hypophysis grows up from below and is at right angles to the long axis of the head (de Beer,1926).

de Beer(1926) questioned as to whether an invagination (Rathke's pocket) or a solid ingrowth is the more primitive *method* of formation of the hypophysis. It is difficult to make a decision. Embryonic conditions must influence the mode of development. Primitively the hypophysis must have been an invagination. The adenohypophysis has evolved from exocrine glandular tissue in the stomodæal ectoderm. Subsequently it became an endocrine organ controlled by the brain (the diencephalic floor). The similarity in the histochemical reactions of the pituitary cells and the mucus-secreting cells of the ectodermal epithelium speaks in favour of the idea mentioned above. Older investigators thought of proliferation leading to a hollow, invaginated gland with an open duct as noted in *Polypterus* or *Cyclostomes*. Recent investigators(Gorbman and Bern,1962) think that the primary phase of proliferation leads to multiple separate acini as found in the pituitary gland of *Myxine*. The open naso-hypophysial duct in *Cyclostomes* may not be the lumen of Rathke's pouch but it is an extension of the naso-hypophysial pit which ventilates the olfactory organ (Wingstrand,1966). That the pituitary of the gnathostome ancestor was hollow is evidenced by the



hollow Rathke's pouch in Amniotes and elasmobranchs and the presence of lumina in different developmental stages in Polypterus, sturgeons, ganoids, Salmo, Clupea, Elops, lungfish, and Gymnophiona (Wingstrand, 1966).

*Comparison with pituitary homologue in invertebrate will help in the understanding of its evolution.* The preoral pit (Hatschek's pit) in the roof of the oral cavity in Amphioxus has been thought to be a homologue of the adeno-hypophysis. This was considered by Goodrich (1917) and de Beer (1926). They pointed out that the right preoral mesodermic cavity in the larval Amphioxus opens out through this preoral pit. In a similar way premandibular coelomic cavities open out through Rathke's pouch in a few vertebrates. In torpedo and in the duck a very comparable state of affairs is seen. The premandibular somites open into the hypophysis in Torpedo and connect with it in the duck. de Beer (1926) said, "The conclusion is irresistible that the hypophysis represents the preoral pit of Amphioxus; the connections which have been called *proboscis pores* in Torpedo and the duck correspond with the similar connection in Amphioxus which is represented in the adult by *Hatschek's pit*. That the connections do not occur more often in the higher forms must be due to the fact that the premandibular somites are differentiated into eye muscles at a very early stage." The *proboscis pore* in the Torpedo and in the duck is formed by a tucking in of the ectoderm to meet the mesoblastic tube from the premandibular somite. This is represented by the lateral lobes of the hypophysis. In Amphioxus the pars tuberalis must be represented by the ectodermal cells of the preoral pit which invade Hatschek's pit and give rise to the so-called rod-bearing cells. This was de Beer's view in 1926. Friedman (1934) was not able to confirm de Beer's theories. In fowl the premandibular cavities disappear early and no *proboscis pores* are formed (de Beer, 1926). In the pigeon Rost (1939) did not observe any such pores. Wingstrand (1966) found that the connections between the premandibular cavities and Rathke's pouch are never established or consist of compact or even discontinuous strings in the majority of cases. An open duct in embryos of *Torpedo ocellata* still speaks in favour of Goodrich's theory.

Barrington (1959) emphasized the potential importance of Hatschek's pit in Amphioxus because there are excellent morphological and embryological grounds for homologising it with the adeno-hypophysis. Its function is said to be the secretion of mucus which helps in trapping the food. It has got a most elaborate cell structure. He said, "Just as a mucus-secreting pharyngeal organ, with powers of iodination, seems to have evolved into a glycoprotein-secreting endocrine gland, so perhaps might a mucus-secreting stomodeal pit, sensitive to passing substances, evolved into another glycoprotein-secreting gland, sensitive to materials reaching it in the blood." The so-called infundibular organ in the brain of Amphioxus is a possible homologue of dorsally situated subcommissural organ of vertebrates because the organ secretes a Rissner's fibre. Previously it



was thought to be a homologue of the neurohypophysis because it contains CAH + substance.

*The cerebral ganglion of ascidians* is situated between the two siphons or incurrent and excurrent openings, dorsal to the pharynx. It is covered by mesenchymal tissue and lies beneath the surface.

*Subneural gland of ascidians (fig. 15.10)*

It is a nonnervous epithelial elaboration of the *excretory canal* which opens into the pharynx. The cavity of the canal branches in the gland in a complicated way. It is intimately related to the cerebral ganglion but separated from it and is usually ventral to the cerebral ganglion but in some ascidian groups it shifts to the dorsal side of the ganglion. The gland is at least partly derived from the neural tube. In salps it is almost wholly formed in this way, but in some ascidians it is partly derived from the roof of the pharynx (Bullock and Horridge, 1965).

The *asymmetric gland* which is in association with the neural gland looks like an endocrine organ. It shows periodic fluctuations in activity which may be related to the *awakening* of sexual activity (Peres, 1943) (from Bern and Hagadorn, 1965).

The ducts of the subneural gland open just behind the papillary zone at the dorsal tubercle. The secretory products are not a liquid substance but *actual cells*, and the gland is not one of internal secretion (de Beer, 1926). Though the subneural gland apparently seems to be homologous with the pituitary, this can hardly be the case and it may be regarded as peculiar to the Urochordata. Extracts from it do not produce any of the effects obtained from the pituitaries of all Craniates so far tested (de Beer, 1926).

Some authors regard the gland as a homologue of the hypothalamus or the neurohypophysis. Others regard it as a homologue of the adenohypophysis because of its partial development from the oral roof. The ciliated funnel formed by the oral ectoderm grows inwards and meets the duct(s) from the gland. This is thought to be a true homologue of the adenohypophysis.

"A homology of the complex as a whole with the pituitary is, at any rate, not excluded, if it is considered how near the adenohypophysial ectoderm is to the future hypothalamic floor (the ventral neuroporic lip) in early vertebrate embryos (Wingstrand, 1966)". Melanophore-dispersing activity, vasopressor and oxytocic activities as determined in the subneural gland-cerebral ganglion complex by earlier investigators have been criticized recently (Dodd, 1959). A gonadotrophic function of the complex obtained by earlier investigators could not be corroborated by Dodd (1959). However, spawning is regulated by the neural





gland of *Ciona intestinalis* and it is the seat of a circadian rhythm, at the morphological and physiological level (Georges, 1978). Spawning inhibitory substance (SIS) has been found to exist. It is produced by the glandular cells during the *epithelial phase* and released during the *mesenchymal phase* (Georges, 1978).

Barrington (1968) said, "It is difficult to avoid the conclusion that the subneural gland complex of urochordates, Hatschek's pit of amphioxus, and the pituitary gland of vertebrates may be to some extent homologues, or may at least show some latent homology, and that the origin of the pituitary gland is bound up in some way with the ciliary feeding habits of protochordate-like ancestors of vertebrates".



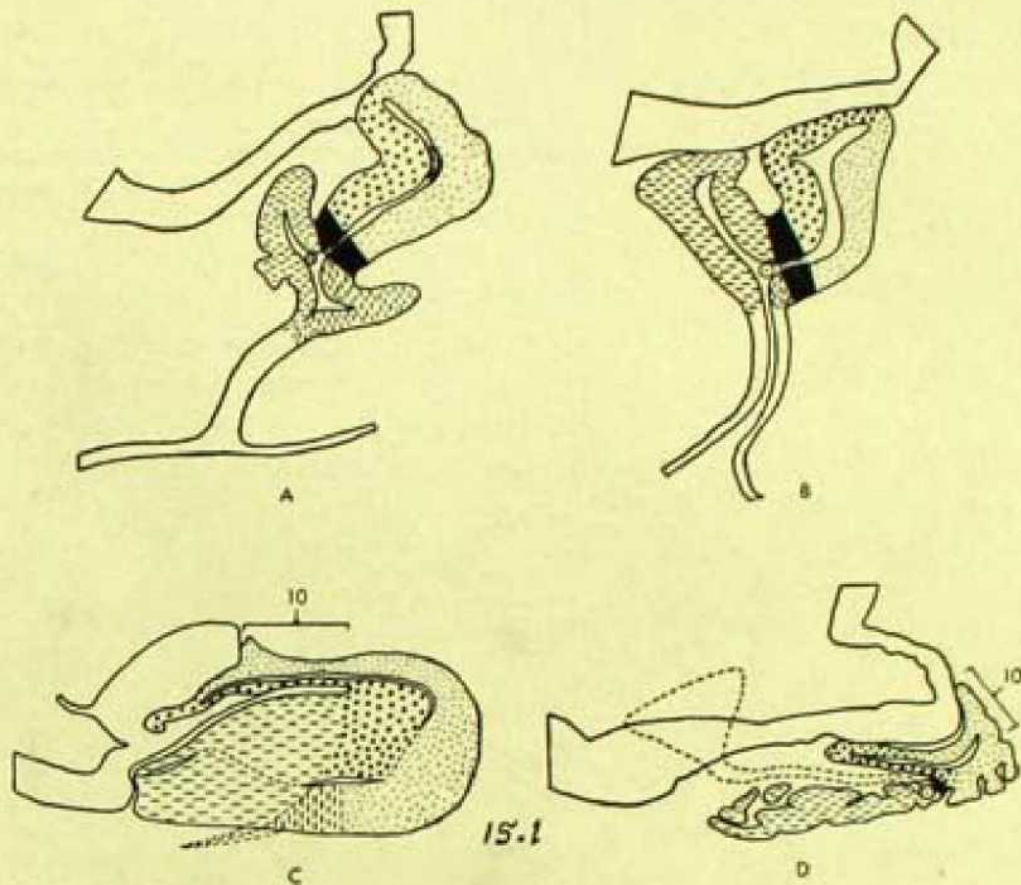


Fig. 15.1. Diagrams showing the development of the adenohypophysis in reptiles.

A. *Vipera berus*, embryo TL 45mm (tail included). The position of the ring marking the lateral lobes is very approximate.

B. *Lacerta muralis*, embryo 4.5mm. After Woerdeman (1914, fig. 22).

The position of the lateral lobes indicated according to the descriptions of Woerdeman.

C. *Bitis arietans*, adult. Structures not present in the section are indicated by broken lines (the probable course of the lumen and the epithelial stalk). As the limits of the different areas are somewhat uncertain, Wingstrand has not marked out the black belt between oral and aboral lobes. 10 = pars intermedia.

Fig. 15.1. Caption Contd. to next page.





Fig. 15.1. Contd. from previous page.

D. *Anguis fragilis*, embryo TL 80mm (tail included). The course of the pars tuberalis which is not present in the median section, has been indicated by broken lines. The limits between the rostral and caudal sides of the oral and aboral lobes respectively could not be ascertained and the figure is, therefore, a little simplified.

For further explanation see fig. 14.1.

(From Wingstrand, 1951. Courtesy of Professor K. G. Wingstrand).

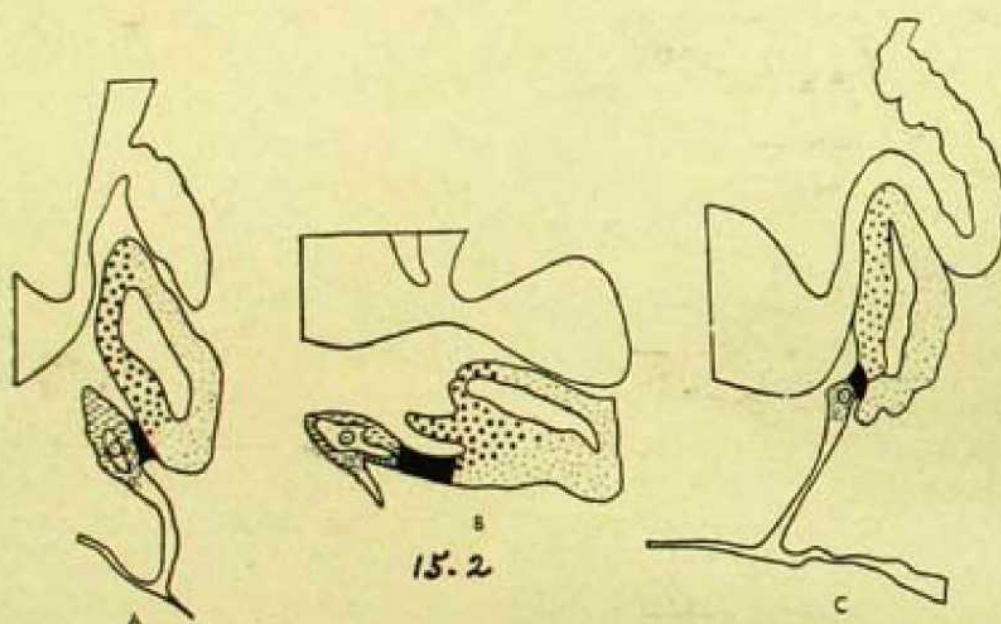


Fig. 15.2. Diagrams showing the early differentiation of the hypophyseal anlage in some rodents

A. Embryo of rat (*Mus norvegicus*), 11.5mm. After Woerdeman (1914, fig. 5). Compare also Schwind (1928, fig. 8).

B. Embryo of rat, 13.5mm. After Woerdeman (1914, fig. 6). Compare also Schwind (1928, fig. 10).

Compare also Schwind (1928, fig. 10).

C. Embryo of guinea pig (*Cavia cobaya*), CR 12mm. Wingstrand's collection.

For explanations see fig. 14.1.

(From Wingstrand, 1951. Courtesy of Professor K. G. Wingstrand).



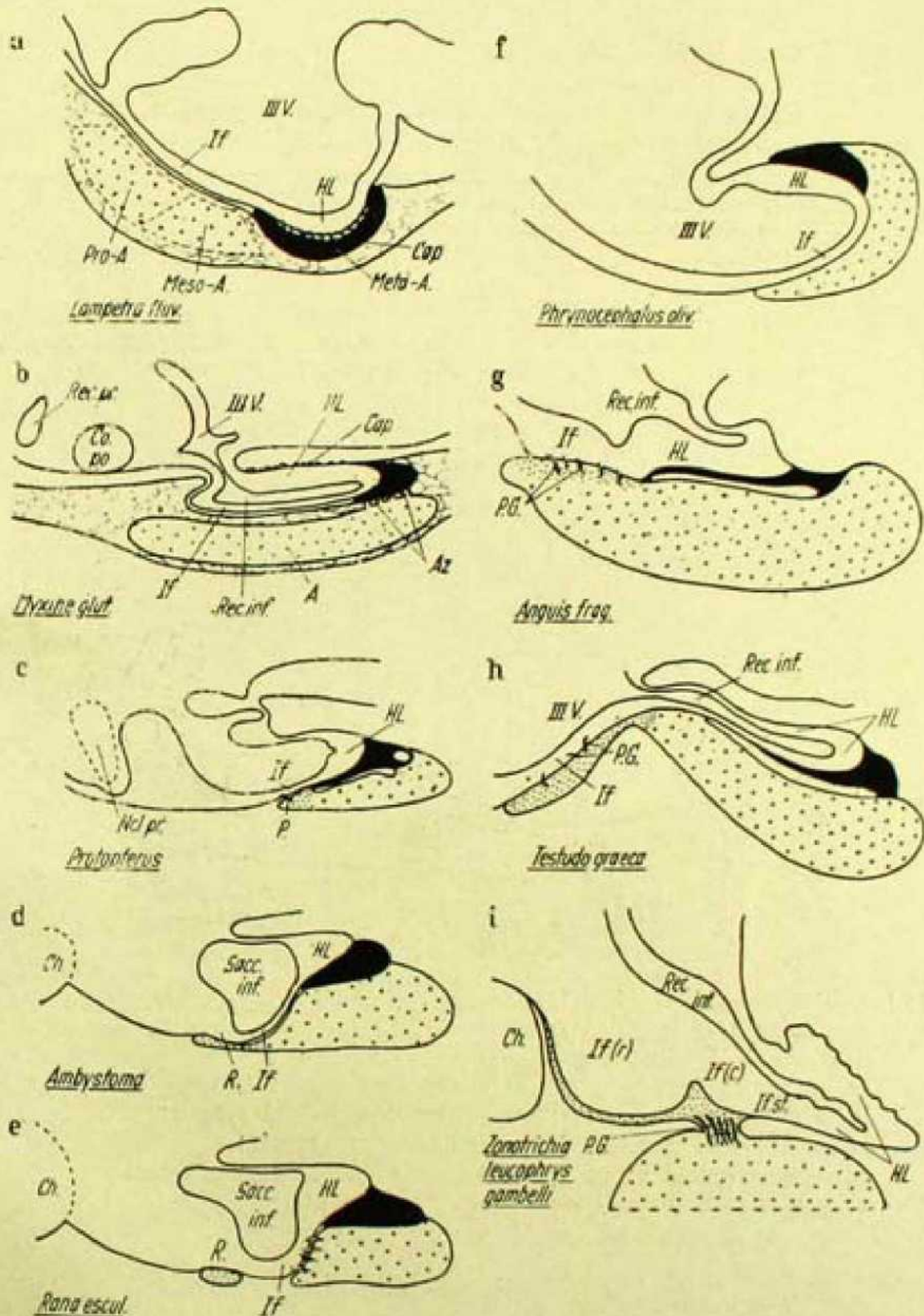


Fig. 15.3. a-i. Schematic median sagittal section through the hypophysis of different vertebrates. Anterior lobe marked with big dots, pars infundibularis with fine dots and pars intermedia is black. HL = posterior lobe; If=infundibulum. a = *Lampetra fluviatilis* (after Adam, 1959). Pro-A=pro-adenohypophysis; Meso-A=meso-adenohypophysis; Meta-A=meta-adenohypophysis; Cap = capillaries between posterior lobe and meta-adenohypophysis. b = *Myxine glutinosa* (after Adam, 1959). A = adenohypophysis; Cap = capillaries on the surface of the posterior lobe; Rec. inf. = Recessus neurohypophyseus;



Fig. 15.3. Contd. from previous page.

Co. po. = post-optic commissure; Rec. pr. = preoptic recess.  
 c = *Protopterus* (after Wingstrand, 1956). P = Portal vessels.  
 d = *Ambystoma*. e = *Rana esculenta*. f = *Phrynocephalus olivieri* (Lacertilia). g = *Anguis fragilis*. h = *Testudo graeca*.  
 i = *Zonotrichia leucophrys gambelii* (after Oksche, 1961).  
 If(r) = rostral part of the infundibulum (Zona externa with Gomori-positive nerve fibre-endings);  
 If(c) = caudal part of the infundibulum; If. st = Infundibular stalk. P.G. = Portal vessels. There is no intermedia. (From Diepen, 1962. Courtesy of Professor R. Diepen and Springer-Verlag).

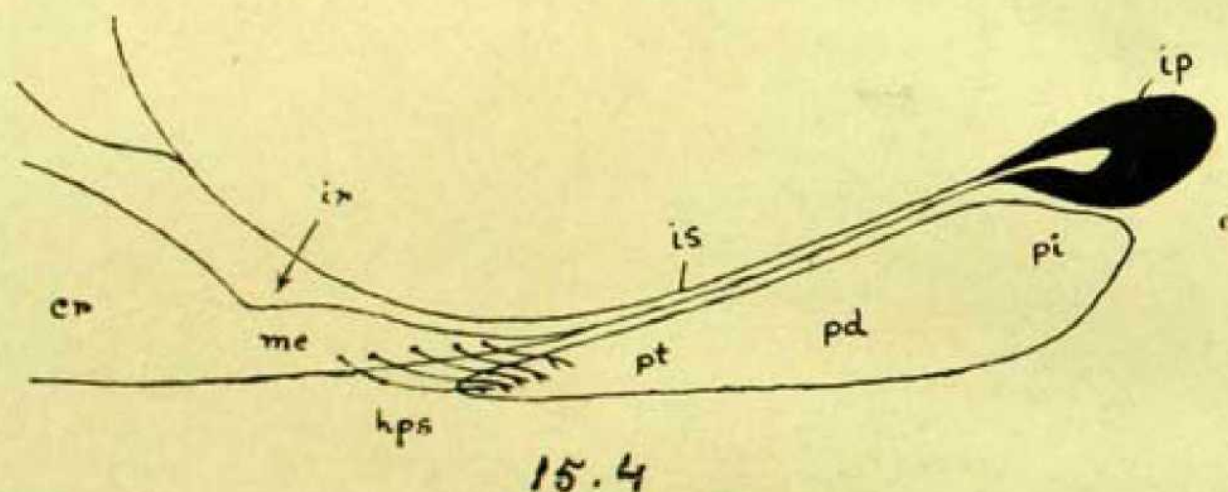


Fig. 15.4. Semidiagrammatic midsagittal section through the pituitary complex of the amphibian (Gymnophione), *Schistomepum thomense* showing the different subdivisions of the hypophysis.

cr = chiasmatic ridge; me = median eminence;  
 ir = infundibular recess; hps = hypophysis-portal system;  
 is = infundibular stem; ip = infundibular process;  
 pt = pars tuberalis; pd = pars distalis; pi = pars intermedia.  
 (Modified from Professor H. Kuhlenbeck, 1973).



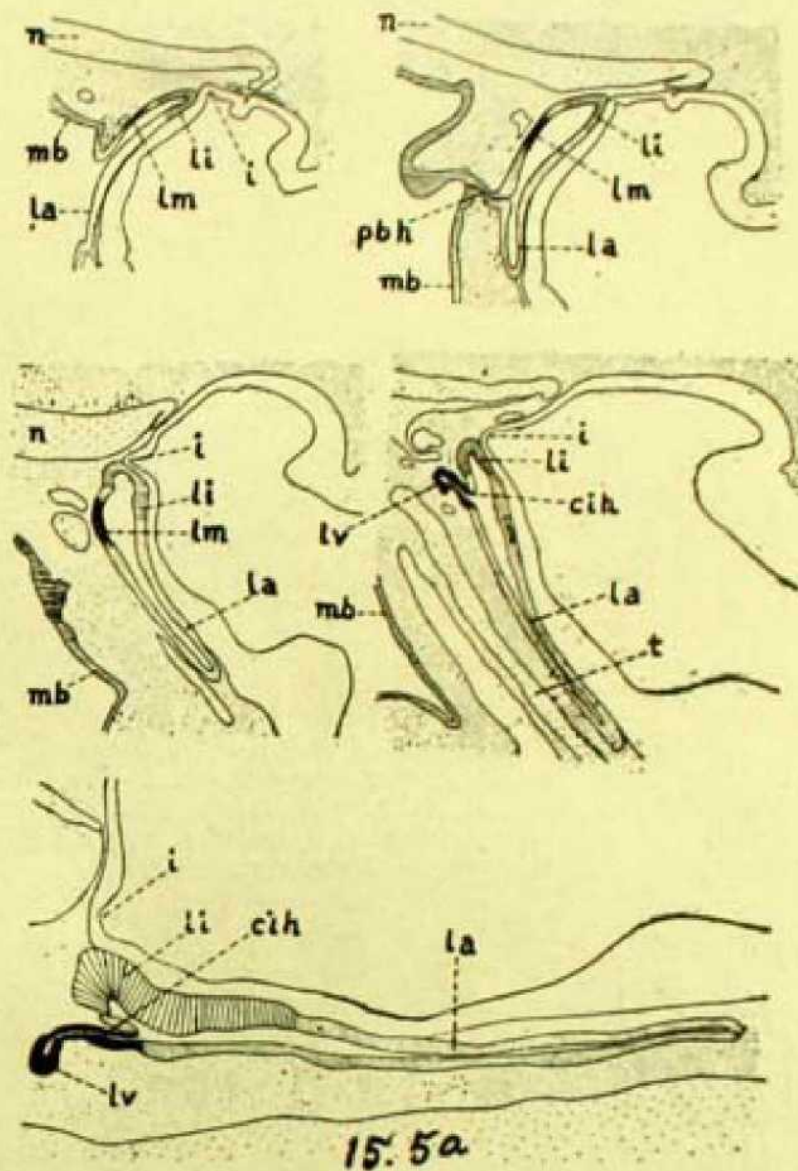


Fig. 15.5a. Scheme of early development of the hypophysis of *Squalus acanthias*. cih, interhypophysial canal; i, infundibulum; La, anterior hypophysial lobe; li, intermediate lobe; lm, middle lobe; lv, ventral lobe; m.b, buccal mucosa; n, notochord; p.b.h, bucco-hypophysial peduncle; t, trabecula (After Baumgartner). (Courtesy of Masson et Cie.)



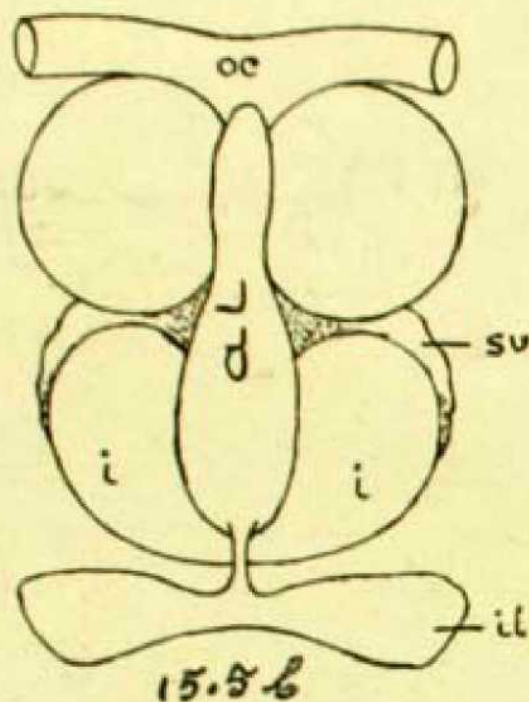


Fig. 15.5b. Ventral view of the pituitary of *Squalus* (after Baumgartner, 1915).

al = anterior lobe (pro- and mesoadenohypophysis),  
i = intermedia, il = inferior lobe, sv = saccus vasculosus.

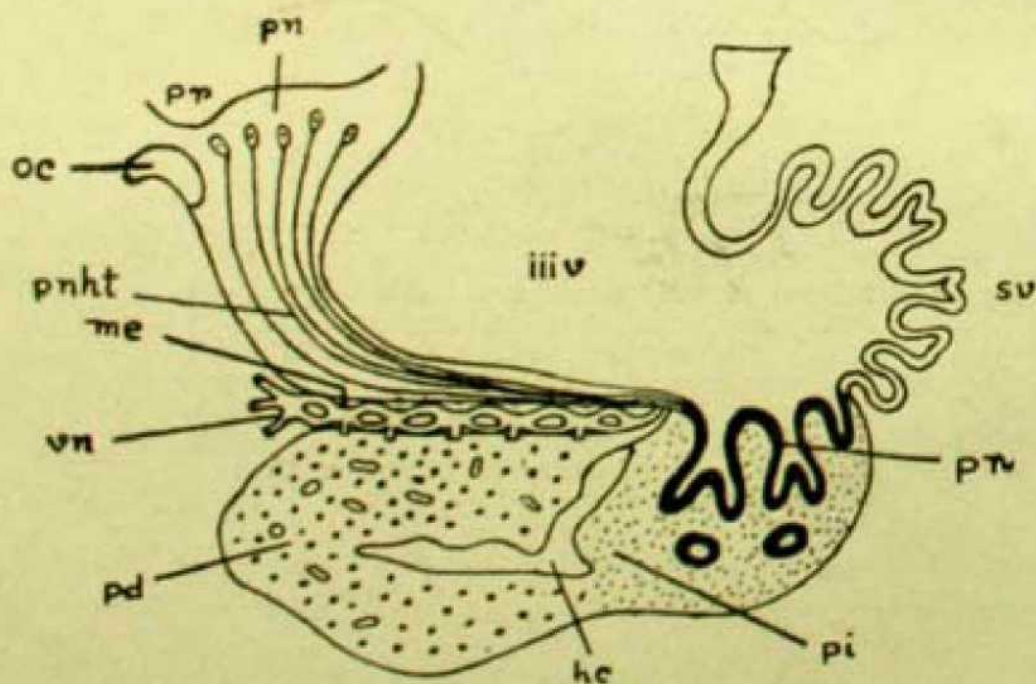


Fig. 15.6A. The pituitary of *Acipenser*. hc = hypophysial cavity; me = median eminence; oc = optic chiasma; pd = pars distalis; pi = pars intermedia; pn = preoptic nucleus; PN = pars nervosa; pnht = preopticoneurohypophysial tract; pr = preoptic recess; sv = saccus vasculosus; iii v = third ventricle; vn = vascular network (From Professor A. L. Polenov).



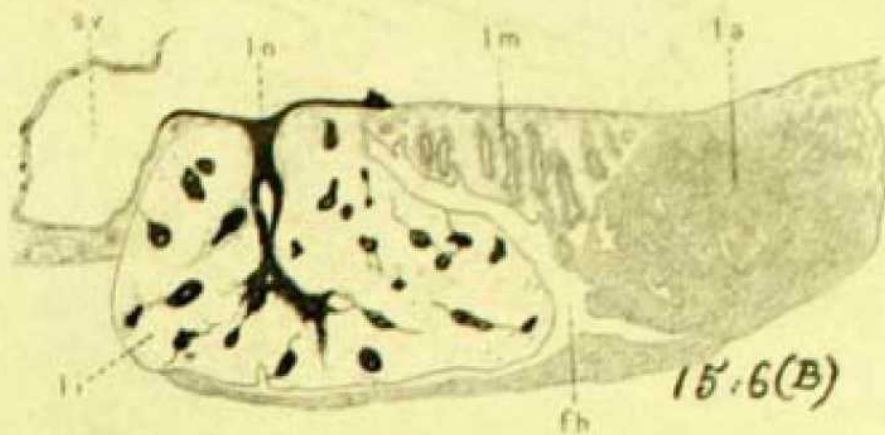


Fig. 15.6B. Schematic diagram of the structure of the hypophysis of *Acipenser sturio*. La = anterior lobe; l.i. = intermediate lobe; l.m. = middle lobe; l.n. = neural lobe; f.h. = hypophysial cleft; s.v. = saccus vasculosus (After Kerr). (Courtesy of Masson et Cie, Paris).

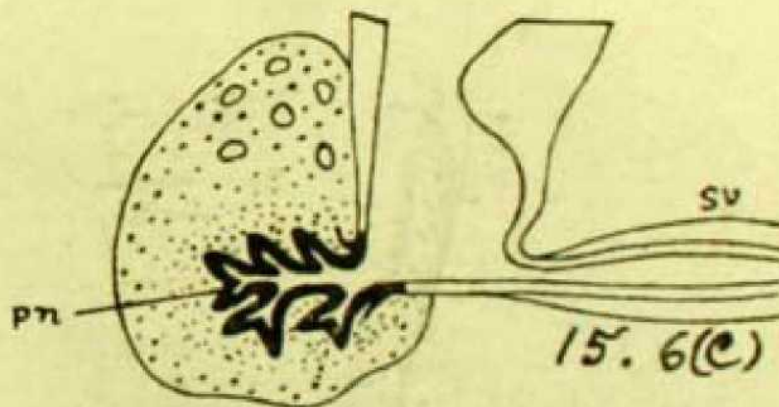


Fig. 15.6C. The pituitary of *Amia*. pn = pars nervosa; sv = saccus vasculosus. (From Professor K. G. Wingstrand).



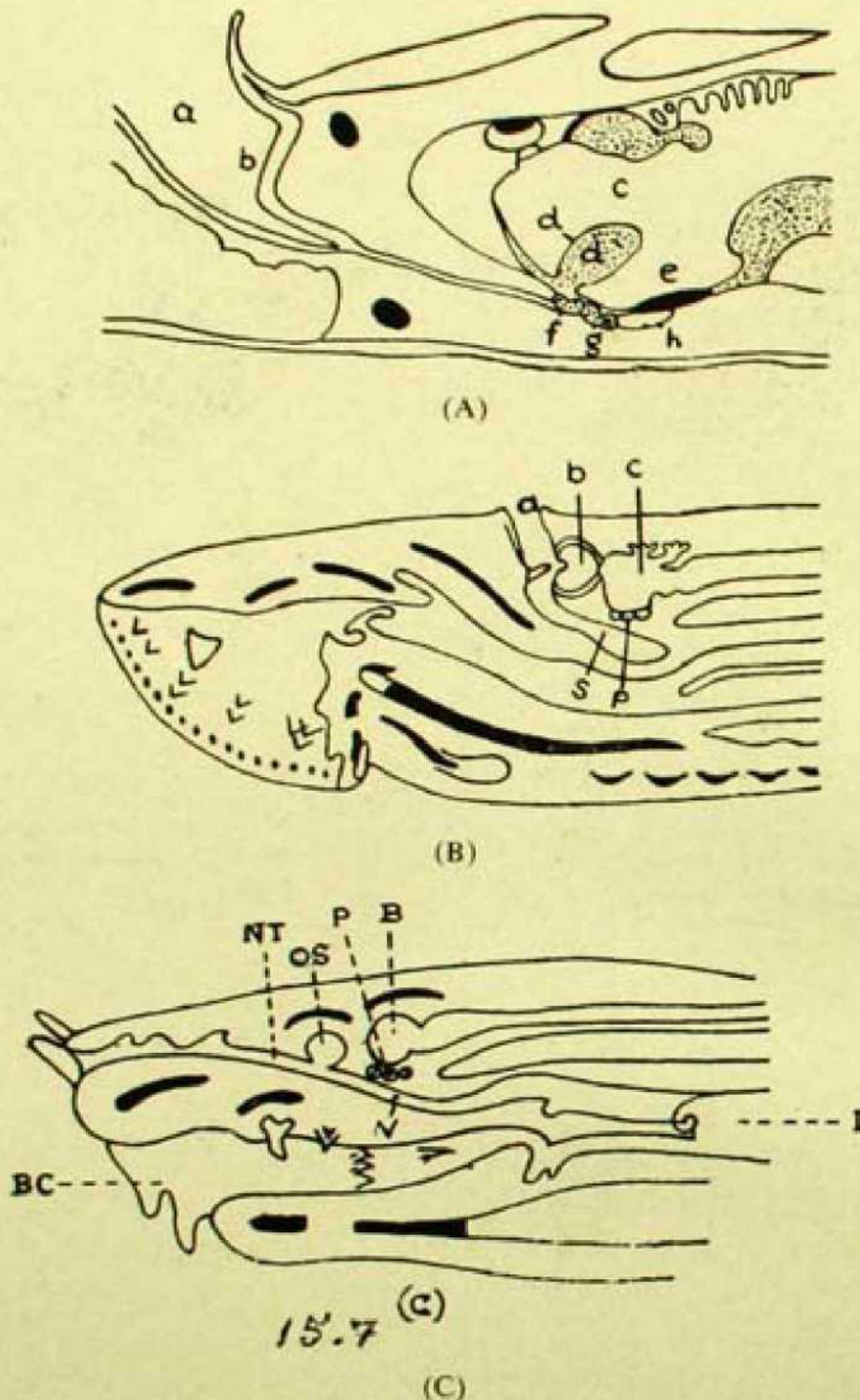


Fig. 15.7. Median sections of the dorsal head region of a larval *Petromyzon*(A), and adult *Petromyzon*(B). a = nasohypophysial pit, b = olfactory organ, c = brain, d = optic chiasma, e = neural lobe, f = proadenohypophysis, g = mesoadenohypophysis, h = metaadenohypophysis, p = pituitary, s = nasohypophysial sac.

(C) = *Myxine glutinosa* (median section of the dorsal head region). BC=buccal cavity, B=brain, NT=nasal tube, N=nasopalatine canal, I=Intestine, OS = Olfactory sac, P = pituitary. (A and B-redrawn after Wingstrand,1966. (Courtesy of Professor K. G. Wingstrand and Butterworths, London) (C-redrawn after Fontaine,1958. Courtesy of Professor M. Fontaine and Masson et Cie Editeurs).



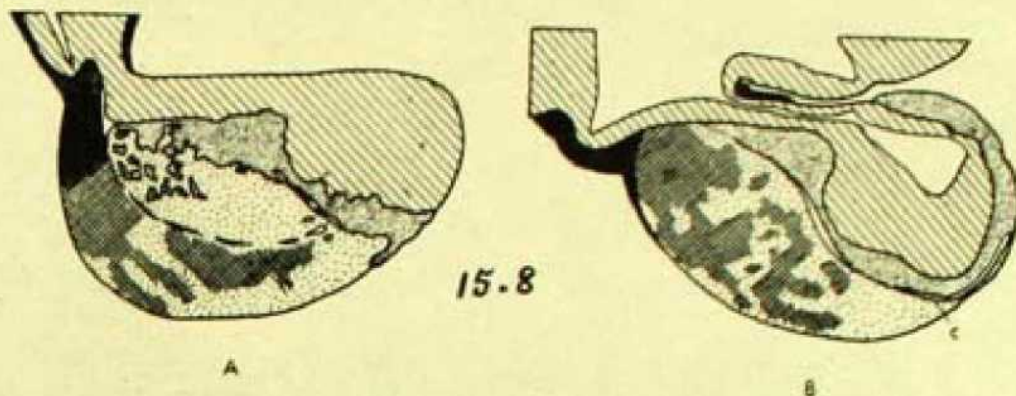


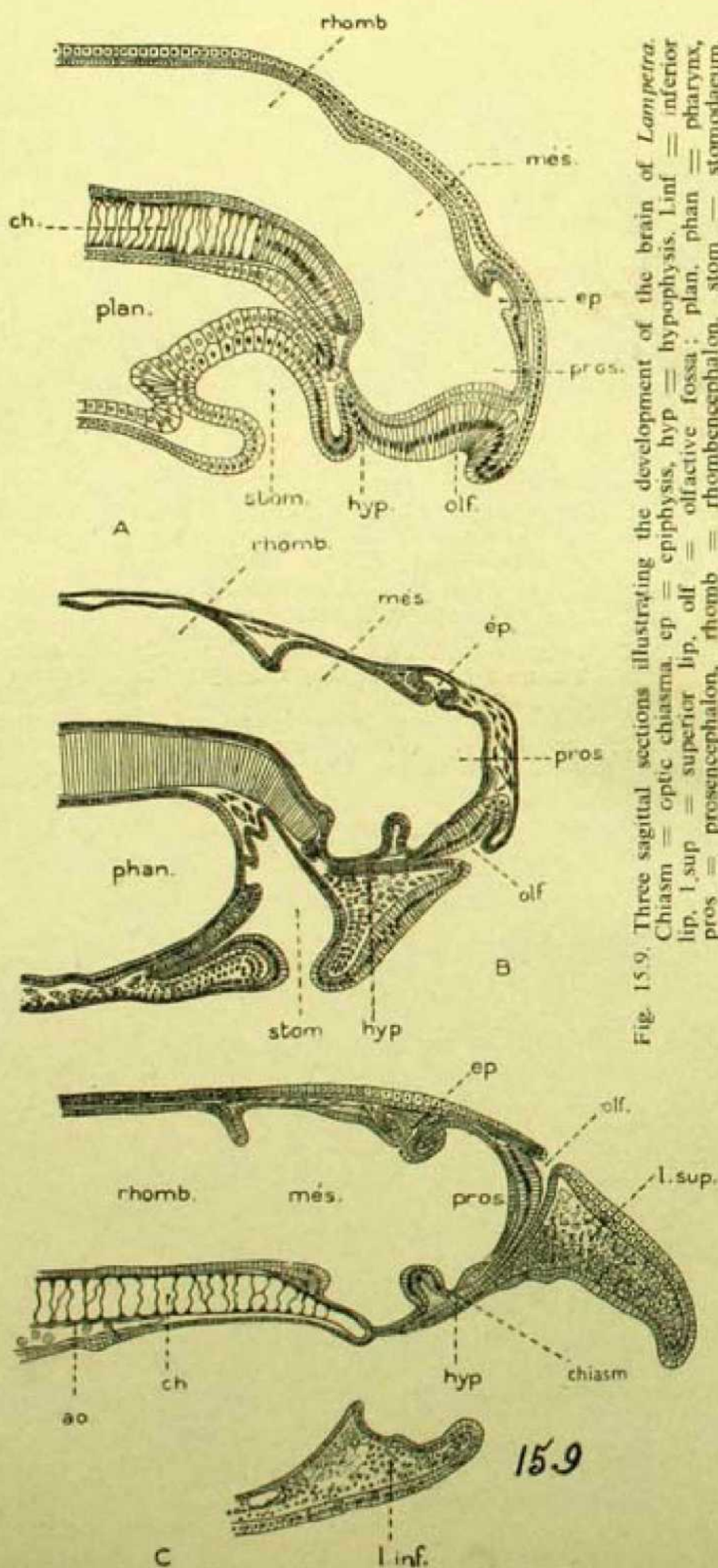
Fig. 15.8. Histological regions in the mammalian pituitary.

Light stippled = pars distalis, dark stippled = pars intermedia, cross-striated = zona tuberalis, black = pars tuberalis, obliquely striated = neurohypophysis. C = residual cleft.

A. Rabbit (*Oryctolagus cuniculus*), median section of the pituitary. After Dawson(1937, fig. 1).

B. Cat (*Felis domestica*), median section. Coll. Hanstrom, Lund. The zona tuberalis is only roughly indicated because it was not very distinct in this specimen, and was partly adjusted so as to agree with Dawson's descriptions and figures (Dawson, 1937; figs. 10-17). (From Wingstrand,1951. Courtesy of Professor K. G. Wingstrand,1951).







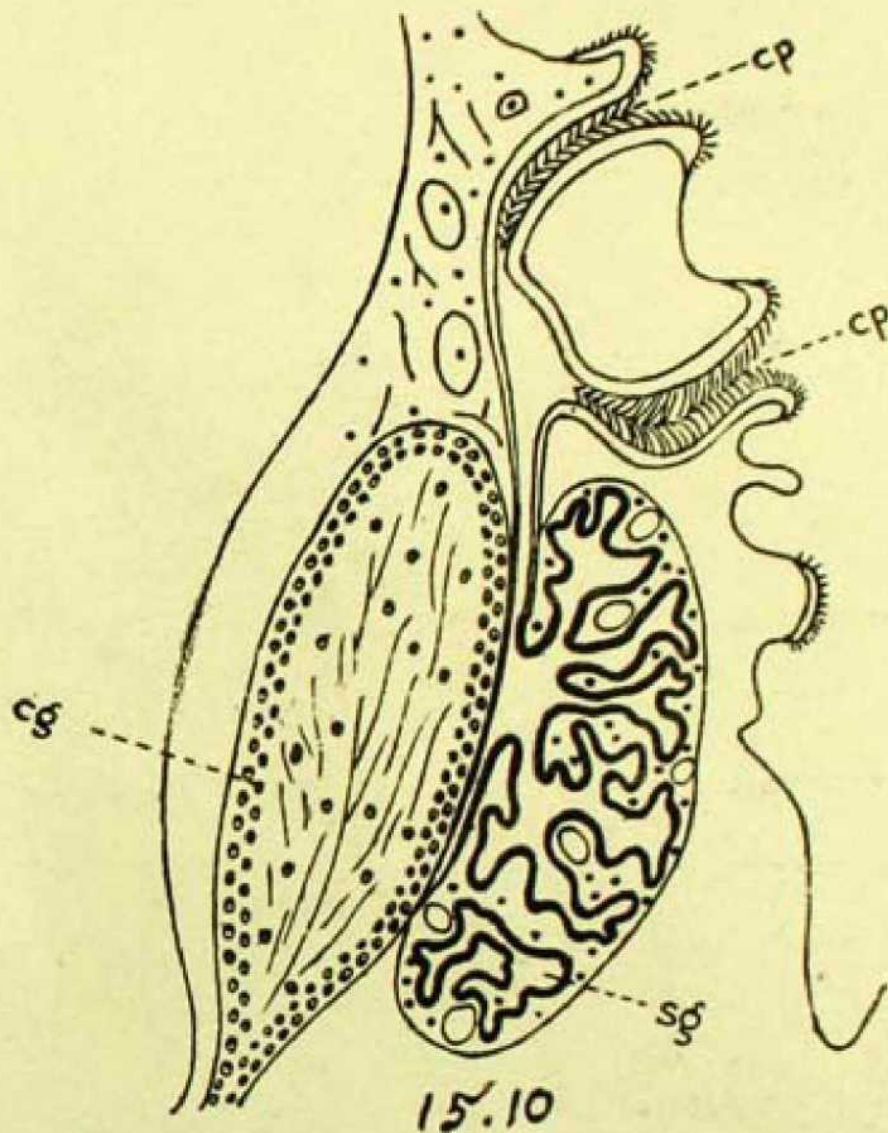


Fig. 15.10. Longitudinal section through the cerebral ganglion and subneural gland of *Ciona intestinalis*. cg = cerebral ganglion, sg = subneural gland, cp = ciliated pit. (Redrawn after Plate, 1922 and Kuhlenbeck, 1967. Courtesy of Professor H. Kuhlenbeck and Academic Press Inc. New York).



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The object of this study by the authors was to find out the effect of hypophysectomy on the store of gonadotropin-releasing hormone (GnRH) in certain parts of the brain as revealed by immunocytochemistry. They prepared the antiserum against synthetic GnRH conjugated with limpet hemocyanin. There was no change in the store of GnRH in the organum vasculosum of the lamina terminalis or in the cephalic segment of the median eminence. Severe depletion was however, noted from the central and caudal (junction with the infundibular stem) segments of the median eminence. GnRH was not detected in the axons of the magnocellular neurons which regenerated during repair of median eminence-pituitary stalk after hypophysectomy.

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The somatotrophs were ovoid to spheroidal and the distribution was quite general in the gland. In the *sex zone* they were scarce. In the male the somatotrophs were larger and more numerous than in the



female. Mammotrophs were polyhedral and were distributed generally in the gland. They were few in the *sex zone*. In the female the mammotrophs were larger and more numerous than in the male. The corticotrophs were few in number. They were small and stellate-shaped. The location of these cells was most commonly near the ventral surface of the gland and the cells formed bilateral centromedial groups in the lateral wings. Thyrotrophs were usually large and polyhedral. They were situated solely in the ventral region of the pars distalis. Gonadotrophs were polyhedral and distributed throughout the gland and they were aggregated in the cephalomedian *sex zone*. Most of the gonadotrophs contained both luteinizing hormone and follicle stimulating hormone.

The glycoprotein hormones consist of an  $\alpha$  and a  $\beta$ -subunit, within a species, and the  $\alpha$ -subunit has a similar structure in LH, FSH, and TSH. The  $\beta$ -subunit is structurally unique and explains the biological and specific immunological properties of the intact hormone. Due to the common  $\alpha$ -subunit there is absence of specificity in immunocytochemical staining. Secondly, "Complete purification of LH, FSH and TSH is difficult so that apparent cross-reactivity may be due to hormonal contamination of the antigen used for immunization, or of the hormone used for absorption in control procedures." Due to the carbohydrate moiety in glycoprotein hormones, the cells secreting them have similarities in histochemical reaction. All basophils are PAS +. Thyrotrophs and corticotrophs of the rat stain with aldehyde fuchsin. The authors stated, "Remaining a problem for future immunocytochemical study of the mouse hypophysis is the possibility that TSH and FSH exist together in some cells. Since most gonadotrophs appear to contain both LH and FSH, the reason why we did not find LH and TSH together in gonadotrophs is not evident. Additional search might have revealed such cells; if present their number must be exceedingly small."

Baker, B. L., and Yen, Y. Y. (1976). The influence of hypophysectomy on the stores of somatostatin in the hypothalamus and pituitary stem. *Proc. Soc. Exper. Biol. Med.*, **151**, 599-602.

28 to 133 days after hypophysectomy of the rat, there was depletion of somatostatin as revealed immunocytochemically from all segments of the median eminence and from the proximal part of the infundibular stem. Consistent change in the somatostatin content of the organum vasculosum of the lamina terminalis (OVLt) could not be demonstrated.

Somatostatin and GnRH are depleted from the median eminence after hypophysectomy. This may be due to degeneration and repair



of the median eminence-pituitary stalk after hypophysectomy. Magnocellular neurons of the supraopticohypophysial system are primarily involved. Somatostatin is not associated with these nerve fibres and so it does not appear in the regenerated areas of the median eminence and stalk. The reduced somatostatin content after hypophysectomy may also be due to interference with the function of other neurons which synthesize and transport somatostatin. The other explanation is that if secretion of somatostatin depends on positive feedback by pituitary somatotropin, the loss of somatotropin by hypophysectomy might reduce the production and secretion of somatostatin.

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The proestrous surge of LH in plasma can be divided into four phases. These are: (1) an initial rising phase, during which time plasma LH rises relatively slowly, (2) a rapid rising phase, during which time plasma LH rises rapidly, (3) a plateau phase, and (4) a declining phase.

Shortly after LHRH infusion was terminated, there was drop of plasma LH and FSH concentrations. "These findings suggested that the proestrous LH and FSH surges require continual or near continual release of hypothalamic LHRH for a period of about three hours followed by a period of diminished LHRH release".

"The LH and FSH surge characterization and stimulation studies ..... revealed two very interesting aspects of the proestrus LH and FSH surges. First, the rapid rising phase of the surges appears to be the result of LHRH self-priming. Second, the declining phase of the surges appears to be the result of both reduced hypothalamic LHRH release and a decreased ability of the pituitary to respond to LHRH". LHRH self-priming and factors responsible for putting an end to the proestrous LH and FSH surges have been discussed by the author.

Dr. Blake also studied morphological and physiological correlates for LHRH self-priming and pituitary refractoriness to LHRH. Continuous infusion of LHRH elevates rat plasma gonadotrophins. By immunocytochemical techniques, LH and FSH gonadotrophs can be identified. The author infused LHRH intravenously into pentobarbital blocked proestrous rats at the rate which simulates the rising and plateau phases of the LH and FSH surges and noted changes in LH-secreting cells with the production of LH surge by utilizing ultrastructural immunocytochemical techniques.



No effect on plasma or pituitary LH-concentration or ultrastructural changes in LH-secreting cells could be observed after phenobarbital injection and a five hour saline infusion, when compared with that of control rats sacrificed prior to the critical period. The basic cell type stained in all control rats was polygonal to ovoid. The granules were evenly distributed throughout the cell or concentrated at one pole.

"At 15, 30 or 60 minutes after the start of continuous LHRH infusion, the concentration of LH in the pars distalis rose significantly despite increases in plasma LH during initial and rapid rising phases". The number of granules in LH cells increased significantly at these times and this feature correlated with LH concentration. These indicate that LHRH self-priming is associated with net increased synthesis and packaging of pituitary LH.

The pituitary became refractory to LHRH and pituitary LH concentration was reduced by one-half to one-third at *five hours* after the start of continuous LHRH infusion (50 ng/h). Marked degranulation was noted in LH-stained cells. It can be concluded that pituitary refractoriness to LHRH is associated with a decreased ability of LH-secreting cells to synthesize and/or package LH. For the pituitary refractoriness to LHRH to develop, there should be a decrease in pituitary LH concentration.

The author tried to correlate morphological changes in FSH-secreting cells during both phases of FSH release during proestrous and estrous.

The author has further explained the 24-hour periodicity in the LH surge and cyclic or daily LH surges. The critical period, activation period and potential activation period have also been discussed in relation to his own previous and present observations. The piece of work also includes estrogen positive feedback and the neural clock, neurotransmitters and LHRH release, and stress and LH release.

### Stress and LHRH release

Ovulation can not be blocked by a variety of acute *stresses*, when the infliction was coincident with the proestrous *critical period*. Similarly, fracture of leg bones, sham ovariectomy, or i. v. injection of 10u of ACTH or 200 $\mu$ g of corticosterone one hour before the onset of the 1400 hours critical period did not affect the LH surge or release of a full ovulatory quota of ova (Blake, 1974, unpublished observation).





*Stressing procedures* just before the starting of the spontaneous LH surge were ineffective to change pituitary LH release. However, if the same procedures are adopted earlier during the estrous cycle, LH release can be blocked or the length of the estrous cycle can be extended. From Blake's laboratory it has been found that implantation of cannulas into the right atrium on the morning of the proestrous did not interfere with LH surge on that afternoon. But cannulation with otherwise ineffective doses of nicotine on that afternoon in proestrous can block ovulation and spontaneous LH surge. "In four-day cyclic rats cannulation of the right atrium during the morning of dioestrous day one but not on the morning of other days of the estrous cycle immediately extended the cycle to five days. This lengthening in the cycle could not be produced by the s. c. injection of 20 u of ACTH in gelatin or breaking the right leg on the morning of diestrous day one. This suggests that *the type of stress rats are subjected to and the reproductive state of a rat both play a role as to whether LH release or length of estrous cycle will be affected by stressful situations*".

*Immobilization stress* in ovariectomized rats suppressed pulsatile LH release and lowered plasma LH concentration. No alteration in the pulsatile patterns in plasma LH could be found after sham ovariectomy four hours before collection of blood, fracture of leg, or intravenous injection of 10u ACTH or 200 mg corticosterone during the collection period. These findings prove that pulsatile LH release mechanism is resistant to many stresses, but the type or degree of *stress* is responsible for the effectiveness for suppression of pituitary LH release. In women *psychogenic stress* causes secondary amenorrhoea and anovulatory cycles. Adrenal epinephrine released endogenously during psychogenic stress or exogenously infused has profound effect on cyclic LH release. Blake concludes, "It is possible that *stress-activation* of the sympathetic nervous system with subsequent adrenal epinephrine release may be involved not only in experimental circumstances of *stress-induced* inhibition of LH release but in psychogenic causes of secondary amenorrhoea and anovulatory cycles".

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Surgical removal of individual lobes of the pituitary of the *Scyliorhinus canicula* was performed separately and in combination. An area of breakdown in the testis was noted after ventral lobectomy and this represented degeneration of late spermatogonia but this effect was only noted when the operation was performed in summer. Removal of other lobes of the pituitary failed to produce zone of breakdown at any time of the year. Weight loss in the testis was noted after ventral lobectomy. Weights of one third of control levels could be achieved within 285 days. Uptake of <sup>3</sup>H-thymidine by the testis is reduced after ventral lobectomy in winter but not in summer. Removal of individual pituitary lobes did not show reduction in plasma testosterone titre. A small but significant fall in testosterone concentration was observed after total hypophysectomy. The testis breakdown after ventral lobectomy is prevented by replacement therapy using a homogenate of ventral lobes but not after injections of mammalian gonadotrophins, HCG and PMSG and androgens.

Dobson, S., and Dodd, J. M. (1977). Endocrine control of the testis in the dogfish *Scyliorhinus canicula* L. II. Histological and ultrastructural changes in the testis after partial hypophysectomy (Ventral lobectomy). *Gen. Comp. Endocrinol.* 32, 53-71.

Ventral lobectomy produces an area of degeneration called *zone of breakdown* in the testis of *Scyliorhinus canicula* L. This consists of an arc of late spermatogonial ampullae in which disintegrating germ cells were being phagocytosed by their associated Sertoli cells. Spermatogonia undergoing the ultimate mitotic division, which converts 8 primary spermatogonia into 16 primary spermatocytes appeared to be the main hormone-dependent stage so far as structural integrity was concerned though earlier mitotic divisions of spermatogonia are also affected, and ultimately cease. Degeneration of mitochondria is rapidly followed by gross cytoplasmic abnormalities, shrinkage of the spermatogonia away from the enveloping Sertoli cell cytoplasm and ultimately there was degeneration of the germ cell nucleus. Degeneration was accompanied





by lipid accumulation in the ampullae and in the interstitium of the testis. These observations were made by histochemistry and electron microscopy.

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In the anencephalic human 7.5-month-old foetal pancreas cells contain immunoreactive somatostatin in the islets as detected by anti-somatostatin. Immunofluorescence was also observed with anti-glucagon and anti-insulin. The cells were smaller than noted in the normal foetus. Fluorescent reaction was never found with anti-somatostatin either in nerve fibres or in nerve endings in the pancreas.

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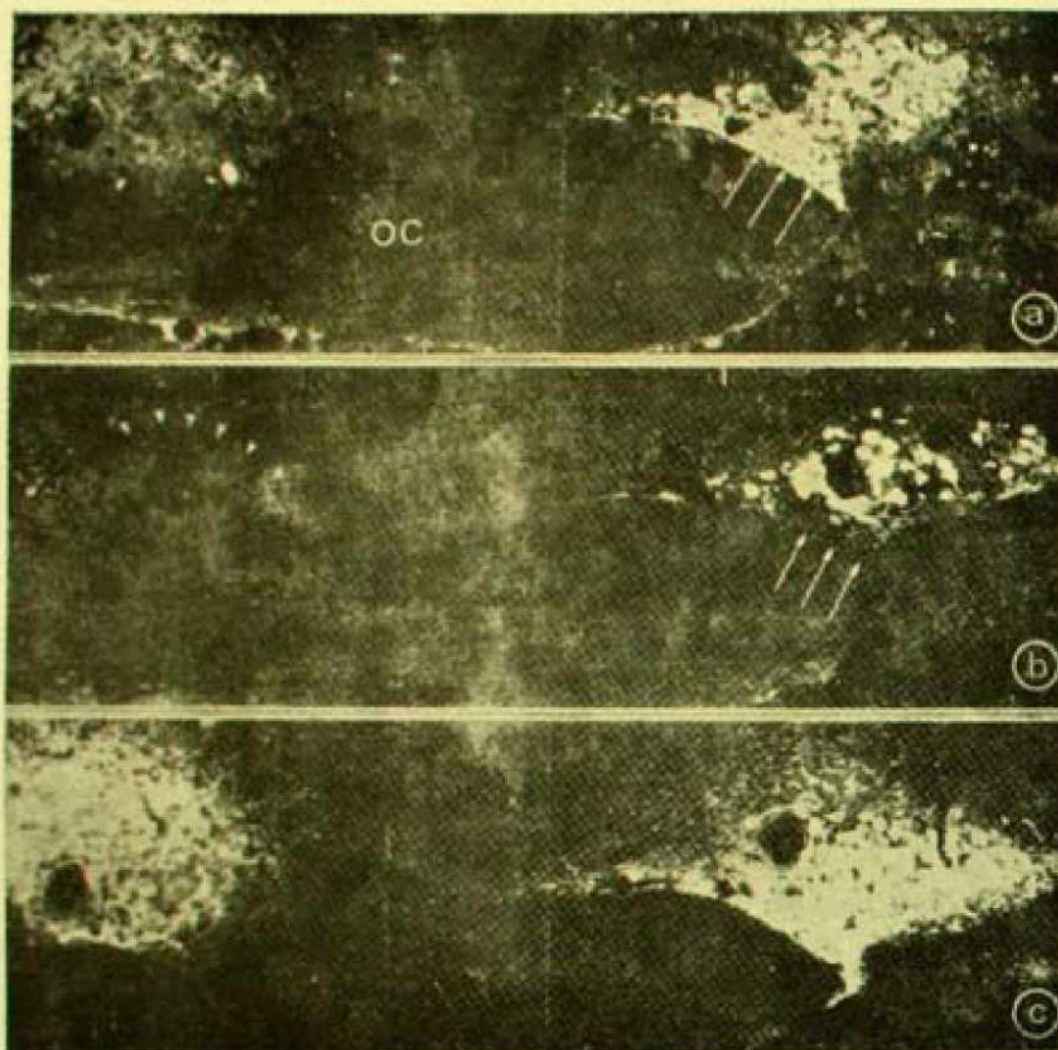


Fig. SB. 1. 1. Immunofluorescence micrographs (mounts) of coronal serial sections of the rat hypothalamus after incubation with vasopressin, oxytocin and neurophysin antisera, respectively. In (a), both the suprachiasmatic nucleus (left) and supraoptic nucleus (right) contain VP (vasopressin) immunoreactive cells and fibres. The group of cell bodies in the supraoptic nucleus immediately adjacent to the optic chiasma (arrows) is localised with this antiserum. In (b), OXY (oxytocin) immunoreactivity is not found in cells or fibres of the suprachiasmatic nucleus (arrowheads), and a different population of cells within the supraoptic nucleus contain OXY. Compare the lack of staining of the cells immediately adjacent to the optic chiasma (arrows) with the intense staining in (a). In (c), note that essentially all cells of the supraoptic nucleus are localised with neurophysin antiserum, and that many of the cells of the suprachiasmatic nucleus stain as well. OC, optic chiasma. Magnification  $\times 120$ . Courtesy of Dr. R. P. Elde, Dr. J. Hughes and the MACMILLAN PRESS LTD.



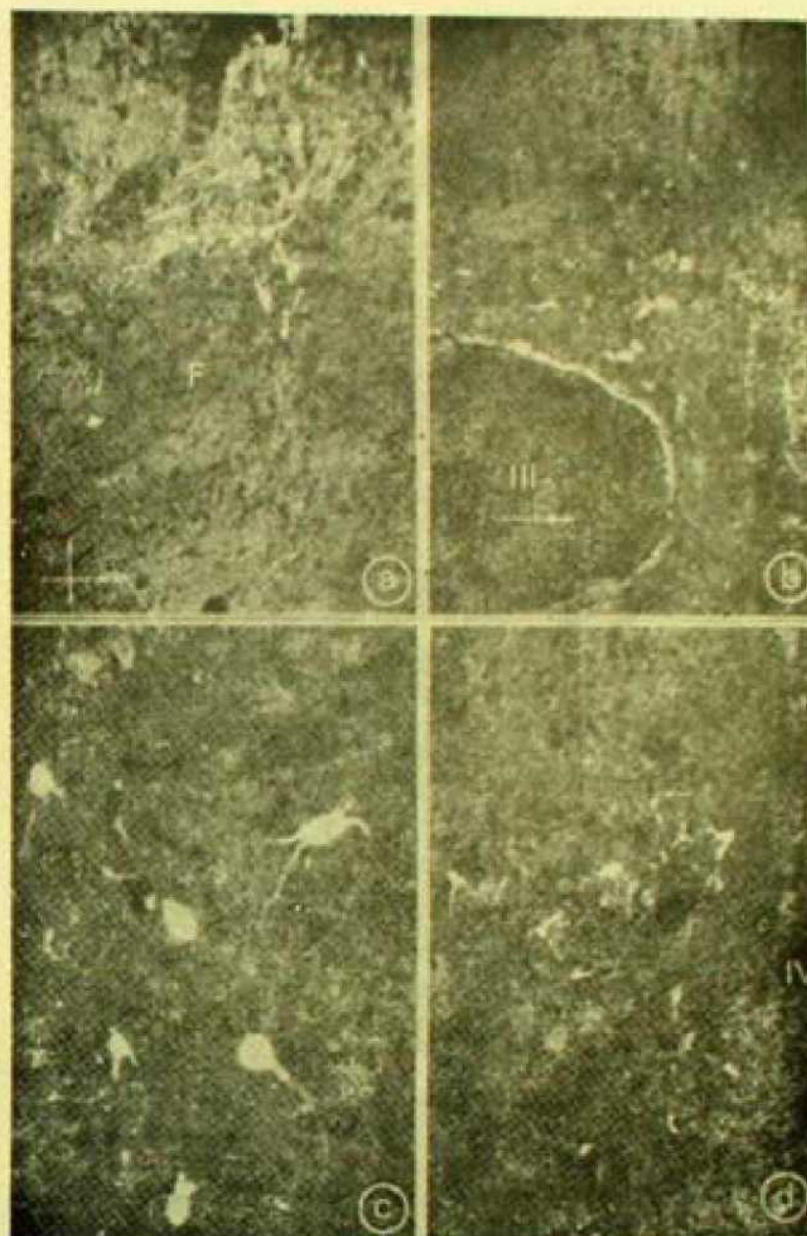


Fig. SB. 1. 2. Immunofluorescence micrographs of coronal sections of the rat perifornical area (a), hypothalamic periventricular area (b), piriform cortex (c) and dorsal tegmental nucleus (d) after incubation with antiserum to methionine enkephalin, TRH (thyrotropin releasing hormone), SOM (somatostatin), and SP (substance P), respectively. In (a) note the ENK (the enkephalins) perikarya medial to the fornix in the vicinity of a large vessel. In (b), TRH-containing cell bodies in the dorsal aspect of the periventricular area are found in small clusters. In (c), several SOM-containing cortical neurons exhibit multiple processes. In (d), note the SP-positive neuronal perikarya in the dorsal tegmental nucleus just ventral to the IVth ventricle. Arrow points medially; double arrow points dorsally. F, fornix, III, third ventricle, IV, fourth ventricle. Magnification  $\times 120$  (a, b, d) and  $\times 300$  (c). Courtesy of Dr. R. P. Elde, Dr. J. Hughes and the MACMILLAN PRESS LTD.



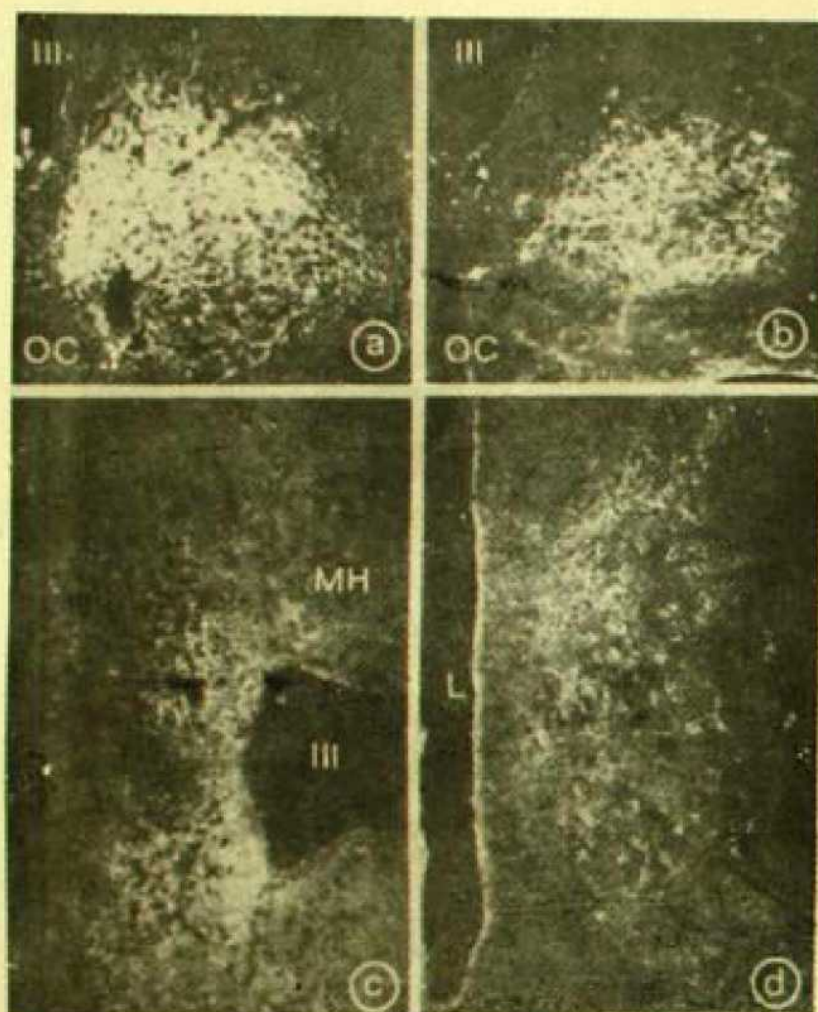


Fig. SB. 1.3. Immunofluorescence micrographs of the rat suprachiasmatic nucleus (a, b), thalamic nucleus paraventricularis rotundocellularis (c) and lateral septal nucleus (d) after incubation with antiserum to NP (neurophysins), SOM (somatostatin), NP (neurophysins) and methionine-enkephalin, respectively. In (a) and (b), note the dense networks of fibres and terminals containing NP and SOM respectively. Note that the NP-containing elements are most prominent in the dorsal and medial aspects of this nucleus, whereas the SOM elements are found in ventral aspects as well. In (c), note the somewhat sparse distribution of NP fibres and terminals in this thalamic nucleus. Several other peptidergic systems also project to this nucleus (not shown). In (d), note the fine, basket-like terminals containing ENK (enkephalins) surrounding some neurons of the lateral septal nucleus. III, third ventricle. OC, optic chiasma, MH, medial habenular nucleus, L, lateral ventricle. Magnification  $\times 120$ . Courtesy of Dr. R. P. Elde, Dr. J. Hughes and the MACMILLAN PRESS LTD.





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In the female rat hypothalamus cell bodies of small to moderate-sized neurons were stained specifically for somatostatin (SRIF) by unlabelled antibody-peroxidase-antiperoxidase immunocytochemical method. SRIF + perikarya were found to be scattered throughout the periventricular nucleus in a limited area which extended from the middle of the optic chiasma to the rostral margin of the median eminence. The same neurons could be detected with either rabbit (R) or guinea pig (GP) anti-SRIF antisera. Positive cell bodies could be more readily detected with GP antibodies because of much less specific background staining than noted with R anti-SRIF. Positive perikarya could not be detected in other hypothalamic nuclei and the ependymal elements were also immunocytochemically negative.

- The authors also studied immunocytochemically the distribution of immunoreactive SRIF in the hypothalami of female Wistar Furth rats bearing the MtTW<sub>1,8</sub> mammosomatotrophic tumor, which produces both GH and prolactin. They report the presence of SRIF+neurons in the hypothalamic periventricular nucleus of such tumor bearing animals.

The present finding of SRIF + perikarya in the nucleus periventricularis hypothalami (NPH) corroborates those of Brownstein *et al.* (1975) where relatively high levels of radioimmunoassayable SRIF was extracted from the same nucleus and after using the same R-ASRIF antiserum as has been used by Elde and Parsons in this present investigation (immunocytochemical studies). In contrast to the report of Brownstein *et al.* (1975), Elde and Parsons (1975) could not demonstrate immunoreactive perikarya in both the arcuate and the ventromedial nuclei of the hypothalamus. It is possible that SRIF + perikarya are restricted to the periventricular nucleus and their axons pass through the ventromedial and arcuate nuclei and terminate in the zona externa of the median eminence.

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In the goldfish endogenous ACTH secretion was suppressed by  $\beta$ -methasone injection. Intraperitoneal injection of lyophilized acid extracts of the hypothalamus or telencephalon of the longnose sucker, or goldfish significantly increased the serum corticosteroid concentration in such an animal. Extracts prepared from sucker or goldfish cerebellum were ineffective in this regard. There is evidence for corticotrophin-releasing factor (CRF) in the sucker and goldfish hypothalamus and telencephalon.

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There was significant suppression of the increase in serum corticosteroid levels (stress response) of goldfish subjected to different types of stresses, having radiofrequency lesions that destroyed both the nucleus lateral tuberis pars anterior (NLTA) and rostral nucleus lateral tuberis pars posterior (NLTP). The different types of stresses used were :

- a) *Sham-injection stress*—The goldfish were netted and a 27-gauge hypodermic needle was inserted 1.3 cm into the peritoneal cavity. The fish were then sampled 15 min. after the return to their holding tank.



b) *Shallow-water stress*—Fish were transferred to buckets containing water 2.0 cm in depth for 15 min. prior to samplings.

c) *Thermal stress*—Fish were kept in warm water (35°) for 10 min. Then they were returned to their holding tank (water temperature 20°) and sampled 30 min. later.

There were no effects on the stress response in goldfish when lesions were placed in the posterior hypothalamus, dorsal telencephalon immediately dorsal to the NLT, or small lesions in the NLT or NLTP. The results suggest involvement of NLT in the control of ACTH secretion in the goldfish and it may be a source of corticotrophin releasing factor.

Lesions of the nucleus preopticus significantly diminished the stress response to a number of stresses and this observation indicates that the neurohypophysial peptides have control over ACTH secretion in goldfish.

Lesions of habenular nuclei increased the stress-response which indicates this area to have an inhibiting action on ACTH secretion.

Fryer J. N., and Peter, R. E. (1977). Hypothalamic control of ACTH secretion in goldfish. III. Hypothalamic cortisol implant studies. *Gen. Comp. Endocrinol.* 33, 215-225.

Significant suppression of the increase in circulating levels of corticosteroids occurring in goldfish after sham-injection stress took place when implantation of pellets containing 0.3 mg of cortisol was made into the third ventricle near the nucleus lateral tuberis (NLT) or into the preoptic recess of the third ventricle adjacent to the nucleus preopticus (NPO). Similar suppression in the stress-response was also observed when cortisol implants (0.5 mg) were made into the third ventricle adjacent to the NPO or the lateral telencephalon posterior to the anterior commissure. There was no effect on the stress-response when cortisol implantations (0.5 mg) were made into the pituitary gland, optic tectum, or lateral telencephalon rostral to the anterior commissure, or into the third ventricle in the posterior or dorsomedial hypothalamus. It may be inferred that the NLT and preoptic-telencephalon regions are negative feedback sides of corticosteroids to suppress ACTH secretion in the goldfish.

Fremberg, M., and Meurling, P. (1975). Catecholamine fluorescence in the pituitary of the eel, *Anguilla anguilla*, with special reference to its variation during background adaptation. *Cell Tissue Res.* 157, 53-72.





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Dr Fuxe and his co-workers presented a lucid summation of more than ten years of histochemical research on the mammalian hypothalamus.

Noradrenergic (NA) terminal systems can control the activity in the LHRH-containing systems via inhibiting inhibitory interneurons, which could contain GABA or enkephalin. NA systems in the medial basal hypothalamus can inhibit the activity in the tuberoinfundibular DA systems.

Certain hypothalamic and preoptic NA nerve terminal systems is important in the phasic LH secretion and may, in part, mediate the positive feedback action of estrogen on LH secretion.

"In order to analyse further the role of DA in the control of LHRH release it will be of importance to demonstrate the DA receptor in the external layer of the median eminence by the use of radioligand for DA receptors such as apomorphine and spiroperidol. Furthermore, recent experiments by Makman demonstrate the existence of a DA-sensitive adenylate cyclase in the median eminence. Once it has been possible to demonstrate





clearly the DA receptors in this region, it can be evaluated whether the potency of DA agonists to bind to the receptor or to activate the adenylate cyclase is correlated with their ability to inhibit the release of LHRH and LH in vivo.

The present in vivo evidence, based on biochemical and pharmacological studies, suggests that the tuberoinfundibular DA systems projecting into the lateral palisade zone participate in the inhibition of LHRH release via an axo-axonic "influence".

Tubero-infundibular DA system to the medial palisade zone may release DA into the primary capillary plexus as a prolactin inhibitory factor. Lateral A12 (DA Cell group in arcuate nucleus) pathway inhibits LHRH. Medial A12 (DA cell group in arcuate nucleus) pathway inhibits prolactin secretion.

The authors discussed the hypothalamic neuron systems which are involved in the control of prolactin secretion. DA released from the median eminence acts as a prolactin inhibiting factor (PIF) and TRH released from the median eminence can have prolactin releasing activity. NA pathways to the hypothalamus can be involved in the control of prolactin secretion, specially when there is stress-induced increase of prolactin secretion. This may be caused by the NA systems which inhibit the activity in the tuberoinfundibular DA neurons and to increase the activity in the TRH-containing systems, 5-HT systems of the hypothalamus is mainly active in lactation, where they exert a marked facilitatory influence on suckling-induced prolactin secretion.

Prolactin can not only activate the DA system to the medial palisade zone involved in the inhibitory control of prolactin secretion, but can also activate the DA pathway to the lateral palisade zone involved in the inhibition of LHRH secretion. So, the inhibition of ovulation found in hyperprolactinaemic conditions may in part be caused via the prolactin-induced activation of this tubero-infundibular DA system.

Friesen, S. R. (1978). Introductory concepts of clinical endocrinology. In "Surgical endocrinology: Clinical syndromes". Ed: Friesen, S. R.; Publisher: J. B. Lippincott Co. Philadelphia, Toronto. pp. 3-17.

Friesen said that, "Because an increasing majority of both entopic—and ectopic-functioning tumors arise from APUD cells, many of which have been shown to be derived from the neural crest of the





neuroectoderm, this body of endocrine abnormalities has been grouped under the term neurocristopathies. The APUD concept, conceived cytochemically, functionally and embryologically, is neither complete nor is it capable of being universally applied to the entire neuroendocrine system. However, there is a clearer understanding of many of the syndromes caused by these endocrinopathies because they have developed under the umbrella of the APUD concept".

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The authors used *in situ* preparations and microtome sections. The supraoptic nucleus can be divided into rostral, median and caudal groups of neurons. The neurons of the paraventricular nucleus are more scattered peripherally and there is a continuity between the SON and PVN. The neurosecretory axons from all these neurons form a consolidated tract anterior to the median eminence. There is extensive ramification of this tract in the neural lobe and they end perivascularly. These neurosecretory axons do not penetrate into the pars inter-media.

Portal vessels are formed by the primary capillary plexus. The neural lobe is vascularized by a hypophysial artery, and a few portal-like vessels formed from the primary plexus. An uninterrupted vascular septum demarcates the neural lobe from the pars inter-media. There is a diffusion of active principles controlling the pars



intermedia from the blood vessels to the gland cells and this is a neuro-vascular mechanism. Direct vascular connection between the neural lobe and pars distalis has also been noticed in this species.

Extension of the neural lobe into the third ventricle occurs and there are a few cases of retention of the remnants of the orohypophyseal duct.

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Ultrastructurally the anti-bSTH positive cells contained oval or rounded secretion granules. The anti-oPRL positive cells had polymorphic secretion granules. In both the cell types some stain on the secretion granules was observed, but reaction products were, in addition, found in the cytoplasm.

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There are eight cell types in the adenohypophysis of *Labeo rohita* as discerned by different tinctorial responses. The rostral pars distalis has 3 types of cells. Thpe 1 is stained with erythrocin, orange G, and acid fuchsin. Type 2 cells are lead haematoxylin positive and an amphiphilic reaction has been noted in response to tri and tetrachrome techniques. Type 3 cells can be stained with PAS, ATh, AB, AF and aniline blue. They are distributed in anteroventral RPD and anterodorsal PPD. In the PPD there are type 3 cells and two other cell types. Classical acidophils (type 4 cells) take up orange G, erythrocin, and acid fuchsin. Type 5 cells are PAS+, ATh+, AB+, AF+, and aniline blue+. This tinctorial affinity is similar to that of type 3 cells, but they are relatively smaller.



The pars intermedia has type 6 and 7 cells which are PbH + and lightly PAS + respectively. The chromophobes are the type 8 cells. They may be immature or degranulated chromophils.

Types 1, 2, 3, 4, and 5 cells are prolactin, ACTH, TSH, STH, and gonadotrophin secreting cells respectively. However, this is to be corroborated by experimental verification.

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With microdissection technique, four circumventricular organs were removed from the rat brain and LHRH and TRH contents were measured. Significant quantities of both releasing factors were found in the subfornical organ, the organum vasculosum of the lamina terminalis (OVLT), the subcommissural organ, and the area postrema. LHRH in the OVLT was 14 ng/mg protein, and 58% of that found in the median eminence.

In other circumventricular organs the concentration of LHRH ranged from 4.2 to 10.2 ng/mg protein. The concentration of TRH in these structures ranged from 0.7 to 1.8 ng/mg protein. The concentrations of LHRH and TRH in the tissue immediately adjacent to the OVLT were nearly 50 times and 4 times less, respectively, than the concentration of these two releasing factors within the OVLT itself.

The circumventricular organs are unique because they are composed of specialized ependyma capable of active pinocytosis, tanycytes, small unmyelinated axon terminals which do not synapse with any effector cells but terminate on capillary walls, small nerve cells which contain neurosecretory granules and small unmyelinated axon terminals arise from the cells, other axon terminals showing synaptoid contacts with small nerve cells and ependymal cells, and a fenestrated capillary bed.

The origin of LHRH and TRH present in the circumventricular organs was not studied by the authors. There are three possibilities regarding their presence :





- a) they may be actively taken up from ventricular CSF ;
- b) TRH and LHRH are made within the circumventricular organs either by tanycytes of the small cells described by Le Breax ; and
- c) they are synthesized elsewhere and transported to terminals in the circumventricular organs by axoplasmic flow.

The circumventricular organs release these factors into peripheral circulation and they may act peripherally, or the releasing factor content of these circumventricular organs, excepting the median eminence is devoid of functional significance through evolution.

Kizer, J. S., Palkovits, M., Tappaz, M., Kebabian, J., and Brownstein, M. J. (1976). Distribution of releasing factors, biogenic amines, and related enzymes in the bovine median eminence. *Endocrinology*, 98, 685-695.

The bovine median eminence was dissected into 8 different subdivisions : rostral, anterior internal, anterior external, middle external medial, middle external lateral, middle internal medial, middle internal lateral, and caudal. Highest concentrations of TRH was found in the middle external medial and lateral subdivisions ; LHRH was concentrated in the middle external lateral and anterior internal subdivisions. Dopamine and choline acetyltransferase were present in highest concentrations in the same subdivisions found to be rich in TRH and LHRH. The distributions of norepinephrine, dopamine-B-hydroxylase, serotonin, tryptophan hydroxylase, phenylethanolamine-N-methyltransferase, glutamic acid decarboxylase, and histamine appeared to correlate poorly with the major distributions of TRH and LHRH. These findings suggest that at the level of the median eminence, central neuroendocrine regulation of TRH and LHRH release may involve an interaction only with dopamine and acetylcholine.

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The authors used synthetic LH-RH (Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>) and superactive analogues, His<sup>2</sup>-Me D-Ala<sup>6</sup> and D-Ser<sup>6</sup>-Des Gly<sup>10</sup>-LH-RH-ethylamide (Superactive analogue I and II). These analogues were 25 to 30 X more potent than LH-RH. LH-RH or its superactive analogues were infused into pituitary portal vessels of adult male rats for 1 minute. Ultrastructural study of the pituitaries was conducted at 1 min, 30 min and 3h after infusion. At 2 min maximal increase of granule release occurred and at 30 min there were dilated cisternae of rough endoplasmic reticulum (RER) and Golgi apparatus (GA). The changes in RER and GA indicate increase in synthetic activity. These changes were noted in gonadotrophs after infusion of LHRH and the superactive analogues but not after saline infusion. Gonadotrophs from analogue infused pituitaries showed a high level of exocytosis and protein synthesis at 3h. Superactive analogues stimulated formation of large vacuoles of RER at 30 min and 3h and these features resembled those seen in *signet ring* cells following castration. These observations were supported by radioimmunoassay (RIA) values of serum LH. These observations provide morphological evidence for a prolonged action of superactive analogues of LH-RH on gonadotrophin secretion and synthesis under physiological conditions.

- Luborsky-Moore, J. L., Steven, J., Poliakoff, S. J., and Worthington, W. C. Jr. (1975). Ultrastructural observation of anterior pituitary gonadotrophs following hypophysial portal vessel infusion of luteinizing hormone-releasing hormone. *Amer. J. Anat.* **144**, 549-555.



Ultrastructural studies following LHRH infusion into a portal vessel have not been done and therefore Luborsky-Moore *et al.* (1975) undertook this problem. Unstimulated gonadotroph from noninfused portion of pituitary shows undistended endoplasmic reticulum. The size of the stimulated gonadotrophs was medium to large and they were located on or near a blood capillary. There were numerous small (130-250 nm in diameter) and large granules (450-650 nm in diameter). These cells contained vesicular endoplasmic reticulum which was usually undilated in control tissue and a large Golgi ring containing a few coated vesicles and granules. Evidence of granule extrusion was there.

Increase in exocytosis was observed in the gonadotrophs, one to three minutes after infusion of LHRH. ER started to enlarge. ER was increasingly distended at 5-10 minutes and more small coated vesicles were present within the Golgi apparatus. Exocytosis was still increased but less than noted at 1-3 minutes. The ER was maximally distended at 15 minutes and the cisternal space was filled with a dense fibrous material. There were new secretory granules within the Golgi apparatus.

The ER was no longer dilated at 30 minutes. There were as many new granules in the Golgi complex as at 15 minutes. The gonadotrophs resembled unstimulated cells at 60 minutes. The ER was not distended, and the Golgi apparatus had only a few new secretory granules. Mature secretion granules filled up most of the cells.

Thus there is morphological evidence for LHRH-induced synthesis in gonadotrophs *in vivo* and it also confirms the occurrence of granule release under physiological conditions.

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Gonadotrophin-releasing hormone (GnRH) and its superactive analogs were administered to normal and aspirin-treated rats and the role of prostaglandins (PG), their active intermediates or the adenylcyclase-cyclic AMP system for gonadotrophin release and/or synthesis was evaluated. Serum LH levels, anterior pituitary malondialdehyde (MDA) content and cyclic AMP (cAMP) levels were followed. More MDA and cAMP were produced by pituitaries stimulated with GnRH or its superactive analog than noted in the controls. Stimulation of the pituitary with the releasing hormones, after aspirin treatment, produced less MDA and lower LH values than the nontreated animals. Aspirin treatment did not significantly lower the cAMP levels. These studies suggest that the activation of the PG biosynthesis and the adenylcyclase-cyclic AMP system are not sequential but two separate physiological events. The active PG intermediates may only be responsible for the release of LH. It is not clear whether the activation of cAMP initiates also the processes preparatory to the synthesis of LH in the endoplasmic reticulum." As pituitary MDA is direct metabolic product from the PG endoperoxide ( $\text{PGG}_2$ ) which gives rise to thromboxane  $\text{A}_2$ , the amount of  $\text{PGG}_2$  formed is more closely related to the amount of MDA than to the amount of any one of the  $\text{PG}(\text{E}_2, \text{F}_{2\alpha})$ , which are end products in the PG biosynthesis and are not directly related to plasma membrane excitatory processes for triggering granule



release). The authors also found that GnRH or its superactive analog stimulates the anterior pituitary with increase in the levels of MDA and thromboxane  $B_2$  ( $TxB_2$ ) in such systems. The superactive analogs used are  $His^2$ -Me D-Ala<sup>6</sup> and D Ser<sup>6</sup>-Des-Gly<sup>10</sup>-LHRH-ethylamide.

With *in vivo* studies the authors further observed that in the rats the analogs (I & II) were 30 times more potent and had prolonged action on the pituitary (3h) than GnRH. "Ultrastructural studies on the anterior pituitary after hypophysial stalk portal vessel infusion of GnRH and the analog (I and II) provided ample morphological evidence for both GnRH and analog induced gonadotrophin-release and synthesis. The prolonged action of the superactive analog (I and II) on the gonadotrophs was also indicated by ultrastructural studies".

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The authors concluded that LHRH controls gonadotrophin secretion and negative and positive feed back actions of gonadal steroids modulate the secretion of LHRH. Gonadal steroids act at CNS and also on the anterior pituitary. These steroids increase or decrease the sensitivity and responsiveness of the gland to LHRH. Responsiveness of the gonadotrophs is increased by the neurohormone. Monoaminergic transmitters primarily control LHRH and gonadotrophin secretion. These transmitters are released at synapses which impinge on LHRH-secreting neurons. Norepinephrine stimulates gonadotrophin release. "Dopamine appears either to facilitate or to inhibit the release of LHRH, according to the steroid milieu existent and possibly according to the degree of dopaminergic activation. Although recent pharmacological





evidence suggests that DA may play a stimulatory role on LHRH secretion during the preovulatory surge of gonadotrophins, DA can also inhibit LH release in some other circumstances, thus reflecting the complexity of its actions on excitatory and inhibitory receptors-".

Serotonin, which is another monoamine, predominantly inhibits LHRH secretion. LHRH secretion under certain circumstances can also be stimulated by acetylcholine and the central amino acid  $\gamma$ -amino-butyric acid (GABA). The role of histamine in the control of LHRH secretion is obscure at present.

"Regarding the mechanism of action of norepinephrine in facilitating LHRH release, it can be postulated that increased NE transmission activates the formation of prostaglandin E, which in turn stimulates the release of LHRH by acting within LHRH neurons located at both the median eminence-medial basal hypothalamus region and preoptic area. Whether PGE acts directly on the LHRH releasing machinery or activates it through the formation of a cyclic nucleotide remains to be elucidated".

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Scanning electron microscopic studies included vascular casts of the pituitary gland, median eminence and hypothalamus from several mammalian species. Observations were also made with light microscopic studies of injected, cleared median-eminen-pituitary specimens and with light microscopic examination of serial sections of injected hypothalamic, median eminence and pituitary specimens employing reflected lighting or epiillumination. Transmission electron microscopy was employed to study long portal vessels on the ventral surface of the rat median eminence.



In these species, the median eminence (infundibular) capillary bed is divisible into an external and an internal plexus. The superior hypophysial arteries supply the external plexus (neurohaemal contact zone) and this is continuous with the capillary bed of the infundibular stem and process.

Blood passes out from the external plexus via three vascular routes :

- a) by fenestrated portal vessels and capillaries to the adenohypophysis.
- b) by capillary connexions to the medial basilar hypothalamus.
- c) by internal plexus capillaries to the ependyma of the median eminence.

"Median eminence vasculature is structurally organized to deliver

- (1) hypothalamic and neurohypophysial peptides to the glandular pituitary via portal vessels,
- (2) hypothalamic and pituitary secretions to the medial basilar hypothalamus via capillaries, and
- (3) hypothalamic and pituitary secretions to distant brain sites through cerebrospinal fluid via ventricular and subarachnoid routes".

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There was enlargement of the cisternae of the RER and Golgi apparatus with formation of secretory granules when TRH (1.25 µg/ml) was added to the incubation medium of rat anterior pituitary fragments in culture. The changes predominantly took place in the thyrotrophs but with some modification they also occurred in the lactotrophs. Similar enlargement of the Golgi apparatus also occurred in the thyrotrophs as well as lactotrophs and somatotrophs after addition of dibutyryl cyclic adenosine 3', 5'-monophosphate to the medium. No apparent change took place in the gonadotrophs with this treatment.

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"The term APUD is an acronym taken from the initial letter of the most constant cytochemical properties of a series of cells whose apparent common function is the synthesis and secretion of peptide or amine hormones".

A=(Inconstant) content of endogenous amines.

P & U=Potentiality for preferential uptake of the amino acid precursors of the two fluorogenic amines, dopamine and 5-hydroxytryptamine.

D=Decarboxylation of the relevant precursors, 3, 4 -dihydroxyphenylalanine and 5- hydroxytryptophan.

Additional letter S=Storage of the amines in or on membrane-bound *endocrine* granules.

Other characteristic features of these cells are :

1. Exhibition of the property of masked metachromasia (and argyrophilia).
2. High content of nonspecific esterases or cholinesterases.
3. High levels of *mitochondrial*  $\alpha$ -glycerophosphate dehydrogenase.
4. The presence of *specific* endocrine-type granules, usually round and 100 to 400 nm in diameter, of various electron densities".

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The neurohypophysis is divisible into infundibulum, infundibular stem, and infundibular process. Median eminence is that part of the infundibulum which forms the floor of the third ventricle. The infundibulum, infundibular stem (or pituitary stalk) and the infundibular process (posterior pituitary gland or neural lobe of the pituitary gland) are continuous with one another.

The superior, middle and inferior hypophysial arteries supply blood to the neurohypophysis. Capillaries from the superior hypophysial arteries supply the infundibulum. The unilaterally or bilaterally situated middle hypophysial arteries supply blood to capillaries in the infundibular stem and upper infundibular process. The middle hypophysial artery has also been called the loral artery (McConnell, 1953), and trabecular artery (Xuereb, Prichard and Daniel, 1954) in the human, and peduncular artery in the rat (Landsmeer, 1951). The infundibular process is supplied by the inferior hypophysial arteries. The retrograde blood flow in the infundibular stem takes place through the dense network of vessels formed by the highly interconnected capillaries in the infundibular stem and infundibular process.

The adenohypophysis is divisible into pars distalis, pars intermedia, and pars tuberalis. In the human foetus, pars intermedia can be easily recognized, but in the adult human it appears largely as clumps of cells interspersed within the pars distalis. On the surface of the infundibular stem and infundibulum, the pars tuberalis is situated as a thin layer of adenohypophysial cells. The well-defined pars intermedia in the rat is essentially avascular and the pars distalis does not ordinarily receive a direct artery supply. The blood





supply is through the long and short portal vessels. So, the blood coming to the sinusoids of the adenohypophysis first passes through the capillaries of the neurohypophysial complex.

In adult animals high rate of secretion of LH after castration depends on the release of LHRH. It also depends on the increased sensitivity of the gonadotrophin-secreting cells in the pars distalis to LHRH in these castrated animals. Gonadal hormone (s) either directly or indirectly suppresses the sensitivity of the pars distalis to LHRH. After gonadectomy the sensitivity of the pars distalis to LHRH increases. It is evident that LHRH is a significant factor which not only causes preovulatory surge release of LH but also it maintains the chronic hypersecretion of LH in the castrated state. Most of the LHRH in the infundibulum is present in axonal terminals. Within synaptosomes, LHRH appears to be compartmentalized in electron-dense particles which resemble dense-cored vesicles. Potassium and calcium help in the rapid release of LHRH from synaptosomes in the incubation mixture. The quantity of gonadotrophin release depends on the quantity of LHRH delivered to the pars distalis and also on the sensitivity of the gonadotrophs to LHRH. Oestrogens and gonadectomy increase the sensitivity of the gonadotrophs to LHRH.

Catecholamines can regulate the pars distalis. The infundibulum has large stores of norepinephrine, a lesser quantity of dopamine and a small quantity of epinephrine. Dopamine acts as physiological inhibitor of prolactin release. Hypophysial portal plasma contains higher quantity of dopamine (between  $10^{-8}$  and  $10^{-7}$  moles per litre) compared to that in arterial plasma of the same animal. Concentrations in both these places were more or less equal for norepinephrine and epinephrine. Infundibulum secretes dopamine selectively.

At the concentration mentioned above, prolactin secretion is inhibited *in vitro*. Highest concentration of dopamine was found in hypophysial portal plasma of rats during pregnancy and lowest concentration was found in intact female rat on pro-estrus, i. e. the day of release of preovulatory LH and prolactin.

High sensitivity of the pars distalis to LHRH around the time of preovulatory release of LH in women may be partly due to a reduction of dopamine secretion by the infundibulum.





Hypothalamic secretion may be regulated by the pituitary through the retrograde blood flow in the infundibular stem and in this way retrograde blood flow transports pituitary hormones to the infundibulum. These hormones include LH, TSH, PRL, ACTH,  $\alpha$ -MSH, and vasopressin.

There are evidences for the fact that PRL (prolactin) stimulates dopamine secretion. Dopamine secretion into the portal blood is highest on the day of estrus—the day following the preovulatory surge release of LH and PRL.

Increased quantity of PRL is secreted in women while they remain amenorrhoeic when they nurse their babies. In women hypogonadotrophic amenorrhoea due to PRL-producing adenomas of pars distalis is due to excessive PRL production.

During pseudopregnancy in female rats there is no ovulatory cycle and this can be compared to amenorrhoea of lactation. PRL increases in a pulsatile manner in the early morning and early evening hours for about 10 days in this circumstance in the female rat. In pseudopregnant rats increased quantity of dopamine is secreted into the portal blood, the quantity of which is comparable to that noted in pregnant rats.

The authors finally tried to integrate hypothalamic and pituitary secretion. The hypothesis relates to dopamine, PRL and LH secretion. They took up the condition of hyperprolactinaemic hypogonadotropism in women with prolactin secreting adenomas in the pituitary. In this situation, blood supply of a localized part of the pars distalis is not through hypophysial portal blood but from vessels (? arteries) in the lower infundibular stem and/or infundibular process. This isolated group of cells is not subjected to the inhibitory action of infundibular dopamine through the hypophysial portal blood and thus the group of cells can act as an explant which is not dependent on hypothalamic control. Explants of cells of pars distalis under the renal capsule can secrete more PRL than an *in situ* gland. If this group of cells in the pars distalis gets blood supply from *middle or inferior hypophysial arteries*, these cells then can act as an explant secreting large quantities of PRL. By retrograde flow, part of PRL reaches the infundibulum through the infundibular stem and dopamine synthesis and release is stimulated and thus the concentration of dopamine increases in the long portal vessels. When dopamine reaches the pars distalis, it inhibits





the release of gonadotrophins. This is achieved by suppression of the responsiveness of gonadotrophs to LHRH. PRL-secreting cells receiving blood from the hypophysial portal vessels can also be suppressed. PRL-secreting cells which receive blood indirectly from the middle and inferior hypophysial arteries are not being acted upon by dopamine from the hypothalamus and so would not be affected by hypothalamic dopamine. This arrangement leads to a condition of permanent hyperprolactinaemia and hypergonadotropism.

The group of cells having blood supply other than through the hypophysial portal vessels may be due to an anatomical variation, or as a result of pressure within the pituitary fossa (due to an adenoma), or change in the direction of blood flow due to vascular resistance after an accident, or due to an adverse psychological experience. These are the conditions which have been offered by the authors to explain the situation.

After transection of the pituitary stalk, there is an alternative source of blood supply to the pars distalis. High concentrations of VIP (vasoactive intestinal peptide) in hypophysial portal blood have been observed in Professor Porter's laboratory and as "VIP causes vasodilatation, it is conceivable that VIP may affect the direction of blood flow in parts of the low pressure vasculature of the adenohypophysis and neurohypophysis". After removal of microadenomas in amenorrhoeic women by surgery, menstrual cycles are normal and pregnancy may happen. When the PRL-secreting cells are too diffusely distributed in the pars distalis, then total removal by surgery is not possible and ergot alkaloids in this circumstance is more helpful.

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The pituitary of *Hydrolagus colliei* is divided into the adenohypophysis, neurohypophysis and an oral Rachendachhypophyse. The adenohypophysis comprises the rostral and proximal pars distalis and neurointermediate lobe. The neurohypophysis is restricted to the pars intermedia only. In the rostral pars distalis there are acidophils, chromophobes, lightly PAS+ cells and amphiphils. The amphiphils can be stained with Heidenhain's iron haematoxylin and lead haematoxylin also. In the proximal pars distalis there are cyanophils (granules are AF+ and PAS+), acidophils, chromophobes and H.Pb+ cells. The perivascular amphiphils in the pars intermedia are H.Pb+, lightly PAS+ cells and there are chromophobes. Few AF+ cells could also be found. Follicular cavities have been noted in all the component parts of the





adenohypophysis and they are probably developed from the hypophysial cavity and these have been noted in the young specimen as a single cavity which extends anteroposteriorly throughout the adenohypophysis.

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The hypothalamic artery gives rise to the primary capillary plexus of the infundibular floor. The portal vessels enter the adenohypophysis and break up into secondary vascular network. In the infundibular floor the dense neurosecretory axonal ramifications are in close contact with the primary plexus. Intense monoamine oxidase activity has also been noted in this region. The infundibular floor of this teleost can therefore be structurally comparable to the median eminence of higher vertebrates.

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Thus hypothalamo-hypophysial portal circulation like tetrapodan type has been convincingly demonstrated in this teleost.

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- Peptidergic cells not previously identified as neurosecretory cells*—They contain substance P, neurotensin, angiotensin, endogenous opioid analgesics (encephalins, endorphins), vasoactive intestinal polypeptide (VIP), gastrin-like and cholecystokinin-like substances and neurotrophic factors-involved in amphibian regeneration (fibroblast growth factor) (FGF).
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The author said, "It is hardly possible to draw a sharp line between stress research as such and work on organs involved in the mechanism of stress reactions. In this treatise I give considerable attention to the types of agents that can act as stressors or modifiers (*conditioners*) of the stress response, the role of nervous and hormonal mediators, and the diseases primarily dependent upon inappropriate reactions to stress, called *derailments of the G. A. S.* On the other hand, only cursory mention is made of the embryology, anatomy, histology and physiology of nerve centers and endocrine glands, the biosynthesis and degradation of hormones and metabolic changes, except insofar as they are directly related to the stress concept. Thus, I do not deal with the embryology of the hypothalamo-pituitary system, the enzymatic mechanisms leading to the synthesis or secretion of corticoids, or the electron microscopic (EM) structure of the median eminence (ME) unless such topics are of particular interest for the interpretation of stress reactions. Even the effects of catecholamines, ACTH or corticoids are considered only in this limited sense".

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The authors reviewed the subject thoroughly. Intense immunocyto-logical visualization of LH-RH fibres in the median eminence has been made by different authors in different vertebrates (frog, toad, duck, cockerel, guinea pig, mouse, hamster, cat, dog, squirrel monkey, rhesus monkey and human foetus). LH-RH-containing granules were of two types. In the axons the granules were 90-120 nm in diameter. In the nerve terminals they were 40-70 nm in diameter. The authors said, "it is, therefore, obvious that the choice of fixative and its delivery to the tissue could greatly influence the ability to detect LH-RH within various anatomical loci. Yet, this consideration can only account for some of the discrepancies concerning the LH-RH perikarya".

For immunocytochemical localization of LH-RH, there are indirect labeled immunocytochemical methods and unlabeled antibody enzyme method. The unlabeled method is more sensitive than the labeled method. Recent investigators have used the unlabeled method. "The unlabeled antibody enzyme method provides a triple amplification of specificity". Even with all necessary precautions and application of the more recent method, visualization of the LH-RH perikarya is not sufficient. The authors thought that the





difficulty in detecting LH-RH perikarya may lie in the fact "that this hormone, like vasopressin and oxytocin, is closely associated with a binding protein which, in the case of LH-RH, alters its antigenic properties ? or it may be that "the antigenicity of LH-RH is masked by peptidization in the form of a prohormone reminiscent of the enkephalin peptide sequence in lipotropin."

Antisera derived from bovine serum albumin (BSA)-conjugates at the terminal glycine position can immunoreact with cell bodies. One such serum is Arimura no. 710.

It requires the more central amino acids for antigenic determinants for binding and does not require either the N-terminal or the C-terminal amino acid for this purpose. Hence, this serum is able to detect not only the bound hormone but also those metabolites of LH-RH in which one or both the terminal amino acids are hydrolysed from the molecule.

In the rat and mouse there are two populations of 'LH-RH' cell bodies, termed as Field I and Field II. These two are quite distinct from each other, both anatomically and immunochemically. The LH-RH Field I is located in the retrochiasmatic and arcuate areas. It can be stained with the antiserum produced against the histidyl LH-RH conjugate (the Sorrentino antiserum F) but not with any antiserum generated against the LH-RH conjugates linked to the glutamyl (i. e. Arimura no. 743) or to the terminal glycyl moiety (i. e. Arimura no. 710). The LH-RH Field II on the other hand, can be stained with Arimura no. 743 and 710, and the cells of this field are located in the medial preoptic and medial septal regions. Further, the cells of this Field II are not revealed by the Sorrentino F antiserum.

The LH-RH cell population in the guinea pig, dog and squirrel monkey does not show the differentiation of Field I and Field II as seen in the mouse and rats. They are stained with both Arimura 743 and Sorrentino F, irrespective of their anatomical location, i. e., medial basal hypothalamic regions or preoptic and septal regions. This may be explained in two ways. It may be that the two fields have merged into a common pool neurons or it is possible that there is a separate 3rd somal LH-RH, having a combination of the structural features of the first two types. A more restricted antigenic reactivity may be the cause of this scarcity of localization





of the perikarya. Since this restriction may be explained by peptidization of LH-RH in which the decapeptide is a part of the molecular structure of a larger protein (that may be termed big LH-RH), it is open to question whether the prohormone was different in the LH-RH of cell types I and II, and the 3rd LH-RH form.

Irrespective of this diversity in the antigenic properties of the LH-RH neurons, the cells, in all the species, have a common tendency to disobey the traditional nuclear boundaries. For example, the Field I cells in the mouse and rats, supposed to occupy the arcuate region, extend laterally beyond the well-defined borders of the nucleus, into the retrochiasmatic and tuberal areas; the Field II cells supposed to occupy the medial preoptic area and medial septal region, extend into the preoptic periventricular area and the nucleus of the diagonal band of Broca.

The distribution of LH-RH fibres is also more widespread than was previously thought. In addition to LH-RH fibres in the ME and OVLT, several hypothalamic and extrahypothalamic projections were found. These include rather prominent collection of fibres in association with the bed nucleus of the anterior commissure, a group of fibres that courses through the periventricular regions of the thalamus, a set of fibres in the ventral portion of the medial mammillary nucleus, a band of fibres running in the habenulo-peduncular tract and terminating in the interpeduncular region of the ventral tegmentum, a group of fibres in the preoptic suprachiasmatic nucleus, and a collection of fibres in the outermost strata of the superior colliculus. The presence of these fibres suggest that LH-RH possesses functions beyond those mediating LH and FSH release by the adenohypophysis.

Whatever may be the extent and course of these extrahypothalamic fibres, it is certain that the most prominent localisation of LHRH fibres lies in the pathways that terminate on the vascular elements of the pituitary portal axis. The major group of these course through the fibrous zone of the ME and undergo branching, wherefrom numerous fibres are sent to the external zone of the ME. This mixing of neuropeptide fibres between the fibrous and external zones is reminiscent of the finding that vasopressin and oxytocin fibres not only travel to the neural lobe as coarse fibres in the internal zone of the median eminence but also as fine fibres in contact with portal capillaries of the external zone. Apart from the





major group, there is a second set of LH-RH fibres that courses over the sulci of the pars tuberalis to enter the external zone of the ME. There is a third group of fibres which enters the vicinity of the portal vessels by coursing rostrally through the floor of the mammillary recess of the third ventricle and contacting the most caudal vessels without passing through the ME.

Two pathways have been suggested to account for the LH-RH fibres travelling to the ME, viz., the tuberoinfundibular LH-RH pathway in the preoptico-infundibular LH-RH pathway, and which of these two is the primary contributor to the fibres of the ME is still a matter of controversy. In the guinea pig, for example, cell bodies have been detected in the preoptic region by Barry *et al.* (1973) and Silverman (1976). While Barry interpreted these as the major contributor to the ME fibres, Silverman holds a different view. She performed a deafferentation of the medial basal hypothalamus and found no remarkable loss of ME fibres. She also demonstrated that lesions of the preoptic area do not reduce LH-RH fibres in the ME, whereas lesions of the arcuate nucleus reduce the ME fibre-staining remarkably.

An antiserum to LH-RH selectively stained the large secretion granules, and to a lesser extent the small secretion granules of the gonadotrophs. "When immunoabsorbed, depleted antisera were used, all gonadotroph staining had disappeared and LH-RH pretreatment evoked no enhancement. To exclude the possibility that the immunoabsorbent was non-specifically destructive to the antibody, anti-<sup>17-49</sup>ACTH was admixed with the anti-LHRH prior to immunoabsorption. The serum mixture stained ACTH cells strongly, and when the sections had been pretreated with LH-RH, gonadotroph staining became equally strong. Upon immunoabsorption with the LH-RH immunoabsorbent, gonadotroph staining of LH-RH pretreated sections had disappeared, but ACTH cells remained unaffected."

Receptor for LH-RH was also seen on the plasmalemma, zona fasciculata and zona reticularis of the adrenal.

A pineal peptide has been recently isolated which may apparently act as luteinizing hormone release-inhibiting hormone. It competes with LH-RH for receptor in the pituitary.





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A decrease in the number of pars distalis fluorescent cells (Day 3) and ultimate disappearance was noted after daily Metopirone injections. There was no marked influence of Metopirone on intermediate lobe fluorescence. A decrease in the number of anti-ACTH binding cells to 30 or 40% was noted 24 hours after adrenalectomy. Total disappearance of these cells was observed 4 days after adrenalectomy. The number of anti-ACTH binding cells in the intermediate lobe was almost identical to that in the control animals. The authors concluded, "a polypeptide, immunologically similar to mammalian ACTH, is simultaneously secreted in both pars distalis and pars intermedia cells in the frog pituitary." The observations also point in favour of the existence of a pars distalis-adrenal feed back mechanism.

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In the bulk-stained preparations neurons of the SON are distributed in three groups : rostral, dorsomedian, and caudal. The PVN is U-shaped, one arm of the U is in close contact with the median component of the SON through scattered neurons. The axons of the PVN extend to the region of the SON and median eminence ; but as they merge with the tract of the SON, their identity is lost. A pair of well-defined median and lateral tracts in the median eminence are in close contact with the vascular net work. The neurosecretory material responds to osmotic stress as found in rats.

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<i>Page no.</i>	<i>line</i>	<i>from</i>	<i>read</i>	<i>for</i>
30	5	top	vasopressin	vasopression
34	Legend to figure 2.3			
	5	top	arteriole	anteriole
35	Legend to figure 2.4			
	9	top	tufts	tuft
	4	bottom	by tuberal veins	by veins
36	Legend to figure 2.5			
	5	top	eminence	eminece
38	7	bottom	form	from
39	2	top	veinules	venules
42	Legend to figure 3.3			
	6	top	cleft	plate
48	6	bottom	disintegrating	disintergrating
53	11	top	elicit	elecit
61	21	top	type V	type IV
61	25	top	granules.	granules
71	20	bottom	occurs	occur
75	6	top	number	numbur
75	11	top	shape.	shape
75	6	bottom	Simultaneous	Simultaneus
76	18	bottom	mammotrophs	mammotrphs
76	12	bottom	lysosomes	iysosomes
76	10	bottom	the	lhe
79	17	top	Hyalinized	Hylinized
80	Legend to figure 6.4			
			read line 3 from bottom for line 4 and line 4 in place of line 3 from bottom	
83	20	top	cells pass	cells
84	6	top	They	The
84	15	bottom	trophs	trops
85	8	top	found.	found
85	11	top	vacuolation	vavuolation
86	12	bottom	increase	increas
89	22	top	cells	celles
89	23	top	size.	size





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Page no.	line	from	read	for
95	17	top	consist	ccnsist
98	9	top	cells	celes
98	21	bottom	vertebrates	verttbrates
101	8	top	development.	development,
102	Legend to figure 6.9 :		to delete line 6 from bottom	
103	22	bottom	glycoprotein	glycoprotcin
103	7	bottom	lobes	lobs
105	Legend to figure 6.10			
	4	top	Atwell	Atwel
	10	top	(1926, Fig. 39).	(1926,) Fig. 39).
	3	bottom	vl	VI
107	Legend to figure 6.11			
	6	top	Guinea pig.	Gcinea pig.
	21	top	Spatz.	Spatz,
109	Legend to figure 6.13			
	14	top	vl	VI
111	7	top	openings.	openings
114	5	top	nsm.	nsm
115	2	top	secretion and pars intermedia.	increase in granulation, activity
115	13	bottom	somes.	somes
115	9	bottom	enzymes.	enzymes
118	17	top	synthesis.	synthesis
118	19	top	change	chage
119	5	top	which	wsich
124	9	bottom	other	othel
124	9	bottom	with	wih
124	6	bottom	persists	persiss
124	3	bottom	autonomy	auonomy
124	2	bottom	after	afer
124	1	bottom	act	acts
126	14	top	filtration).	filtration
126	1	bottom	crinophagy".	crinophagy"
127	11	top	days.	days
130	19	top	region.	region





<i>Page no.</i>	<i>line</i>	<i>from</i>	<i>read</i>	<i>for</i>
133	7	top	dilatations.	dilatations
136	7	top	to be read above line 1	
136	18	bottom	Glycogenic	Glyocogenic
137	5	bottom	Moriarty	Moriarity
138	12	top	Moriarty	Moriaty
140	2	top	horizontal	hoizontal
140	13	top	contains	contain
141	Legend to figure 6.15			
	8	top	140,	140.
147	20	top	stated,	stated.
150	10	top	area.	area
150	12	top	neuro-	neuro
150	5	bottom	dorsal wall.	dorsal wall
151	11	top	axons.	axons
153	8	bottom	effect)	effect
153	7	bottom	testis.	testis
155	18	top	neural	neutral
157	1	bottom	neurohypophysis.	neurohypophysis
160	19	top	Rothwell, 1972 ;	Rothwell,. 1972
161	17	top	cells.	cells
162	14	top	luteinizing	luteinzing
162	15	top	mone releasing hormone (ir-LHRH)	mone (ir-LHRH)
162	18	bottom	tionary	cionary
164	18	top	fuses	fuse
171	Legend to figure 8.4			
	3	bottom	maternal	meternal
	2	bottom	cic	cie
172	19	bottom	<i>radiata</i> inhibition of synthesis	synthesis <i>radiata</i> inhibition of
173			read line 19 in place of line 20 and line 20 in place of line 19 from bottom	
175	4	top	develop-	vevelop-
183	11	top	ventromedial	vetromedial





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Page no.	line	from	read	for
189	Legend to figure 10.4			
	1	top	Rhodeus	Rnodeus
196	12	bottom	aniline	alinine
197	1	bottom	affinity	afinity
201	6	top	<i>mossambicus</i>	<i>mossambieus</i>
203	6	bottom	granule	grenule
204	18	top	to delete <i>and podium</i>	
204	19	bottom	gonadotrophic	gonadotropic
204	17	bottom	stimula-	stimula
205	2	top	LTH	LHT
205	5	top	granulations and they	granulations they
205	16	bottom	prominence.	prominence
208	11	top	<i>Cyprinus</i>	<i>Cyprius</i>
208	15	top	serotonin	serotomin
209	9	top	studied	studid
209	12	bottom	fish.	fish
211	7	bottom	<i>GTH cells</i>	<i>GTH</i>
213	16	top	vitellogenesis".	vitellogenesis"
214	12	top	immunoperoxidase	immunoperoxiadase
217	3	top	S.	So
219	13	top	Ball	Ball,
219	18	bottom	<i>heteroclitus</i> ,	<i>heteroclitus</i>
219	15	bottom	10.8	108
220	4	top	<i>et al.</i> ,1969),	<i>et al.</i> , (1969),
220	Legend to figure 10.7			
	1	top	teleost,	teleost.
221	Legend to figure 10.8			
	3	top	somatotrophic cells ;	somatotrophic cells
222	5	bottom	hydroxytryptophan	hydroxptryptophan
222	2	bottom	complete	ccomplete
223	2	top	areas	aras
223	10	bottom	granules)	granules).
224	19	top	granules	granules





# ERRATA

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Page no.	line	from	read	for
224	10	bottom	microscope.	microscope
226	3	top	<i>Proudenohypophysis</i>	<i>below Part of the pituitary</i>
226	3	top	<i>Acidophil</i>	<i>below cell type</i>
226	15	top	other	othar
227	3	top	<i>Mesoadenohypophysis</i>	<i>below Part of the pituitary</i>
227	3	top	<i>Basophils</i>	<i>below cell type</i>
228	1	top	<i>Metaadenohypophysis</i>	<i>in place of " in column 2</i>
273	16	bottom	synthesis	svnthesis
316	7	top	They	The
331	12	top	to be deleted	
331	20	bottom	adrenocortical	alrenocortical
349	19	bottom	secondary	sencondary
349	11	bottom	number.	number
356	11	top	controls.	controls
xxxvi	lines 10—12 from top to be read above line 11 from bottom			
xxxvii	6	bottom	Add 158, 169—175	
xxxix	Legend to figure SB. 1.2			
	6	top	(thyrotrophin	thyrotropin
	7	top	hormone),	hormone,
	12	top	aspect	aspect

3-2-04